University of Southern Queensland

Faculty of Health, Engineering and Sciences

# Investigation of methane production by anaerobic co-digestion of food waste, fats, oil & grease, and thickened waste activated sludge using Automatic Methane Potential Test System

A dissertation submitted by

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in fulfillment of the requirements of

**Courses ENG8411/ENG8412 Research Project**

towards the degree of

**Master of Engineering Science (Environmental)**

Submitted: October 2017

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**University of Southern Queensland**

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# ENG8411/ENG8412 Masters Dissertation Project

(This is a 3 unit research project in a 16 unit Master of Engineering Science program)

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# ABSTRACT

This project investigated the methane production by anaerobic digestion using Automatic Methane Potential Test System (AMPTS). Food waste (FW), waste cooking oil- Canola oil (FOG), and thickened waste activated sludge (TWAS) were used as substrates for anaerobic digestion in two sets of experiments. Energy harnessed from waste by anaerobic digestion can be used to replace fossil fuels, which release harmful compounds in the environment.

Substrates and inoculum were characterized to find the content of total solids, total volatile solids, chemical oxygen demand, total organic carbon, and total nitrogen. All the substrates were digested individually in the first set of experiments. In the second set, they were combined in different proportions (four combinations) and were co-digested. All the experiments were carried at mesophilic temperature (37˚C). In this study, emphasis was given to the percentage of FOG which can be inhibitory for methane production.

At the end of the first set, it was found that FW generated the maximum methane, followed by TWAS. Very less methane was produced from FOG. Results obtained from the first set established that FOG is not a suitable substrate for anaerobic digestion. From the second set of experiments, it was determined that FOG did not cause inhibition. However, presence of FOG in co-digestion process caused problems which led to decreased yield of methane in all the four combinations. These problems included accumulation of FOG at the top surface of the solution in AMPTS bottles, coating of oil on microbes' bodies and substrates, lack of proper mixing in the bottles, and formation of a thick solution which included all the substrates, inoculum and bio-medium. As a result, in this study, co-digestion did not provide better methane yield than single substrate anaerobic digestion. It was determined by the second set's results that FOG reduces the methane yield if co-digested with FW and TWAS.

# ACKNOWLEDGEMENTS

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# GLOSSARY

| | | |
|---|---|---|
| AMPTS | = | Automatic Methane Potential Test System |
| C/N | = | Carbon to Nitrogen ratio |
| COD | = | Chemical oxygen demand |
| FOG | = | Fats, oil & grease |
| FW | = | Food waste |
| I/S | = | Inoculum to substrate ratio |
| KW | = | Kitchen waste |
| LCFA | = | Long Chain Fatty Acid |
| TN | = | Total nitrogen |
| TOC | = | Total organic carbon |
| TS | = | Total solids |
| TWAS | = | Thickened waste activated sludge |
| VS | = | Volatile solids |

# CHAPTER 1: INTRODUCTION

## 1.1 INTRODUCTION

Organic waste produced from domestic, industrial and agricultural activities is increasing at a fast pace owing to growth, development, globalisation and increasing competition. Disposing off this waste is becoming a major concern for different industries as it causes pollution if left untreated. Also, burning of fossil fuels for energy is also a threat to the environment because of increasing carbon dioxide emissions in the atmosphere. Anaerobic digestion is a way of treating and generating cleaner energy from the waste. It is a process by which organic waste is decomposed by microbes in an oxygen-free environment. In this process, methane and stabilised compost are generated. Methane can be used as a replacement for fossil fuels as it is cleaner. Ward et al. (2008) state that anaerobic digestion can be applied to a variety of feedstock including industrial and municipal waste water, agricultural, municipal and food industry wastes. Some of the advantages offered by anaerobic digestion are also mentioned:

- Less biomass sludge is produced by this process in comparison to other processes, e.g. aerobic digestion.
- It produces a residue which can be used as a soil conditioner.
- It is an effective pathogen removal technique.
- Biogas is produced in the process which is a carbon neutral energy source. Carrying out anaerobic digestion in sealed container, will trap the methane gas, which is a greenhouse gas. Also, methane can be used to replace the fossil fuels, which on burning produce carbon dioxide. On the other hand, on combustion, methane releases carbon neutral carbon dioxide which enters the carbon cycle.

- Reduces the odour problems
- Net generation of energy
- Lower land requirements in comparison of other methods, e.g. composting

Xie et al. (2017) state that a recent and notable development in anaerobic digestion is to co-digest two or more substrates together. There are some problems associated with single substrate digestion such as lack of micronutrients, imbalanced C/N ratio, a higher biodegradable fraction etc. These inherent problems can be overcome by co-digestion. Optimal mixture composition between the substrates can be investigated by measuring specific methane production rate. This project aimed to investigate the methane production by the anaerobic co-digestion of food waste, fats, oil and grease, and thickened waste activated sludge. Special emphasis has been made to the percentage of the fats, oil and grease in total substrate as after a certain quantity it inhibits the production of methane (Long et al. 2012).

## 1.2 BACKGROUND

With the growing concern for the disposal of waste from various industries, including food industry, in this project food waste was chosen to be one substrate. Another issue which the food industries are facing is the disposal of fats, oils and grease. Williams et al. (2012) state that fats, oils and grease deposits in sewers are a major problem as they could cause sewer overflows, leading to environmental damage and health risks. On the other hand, FOG enhances the methane production if used as a substrate in co-digestion process. Long et al. (2012) state that if fats, oils and grease is co-digested with activated sludge from waste treatment plants, the methane production is increased by 30% or more, which may allow waste water treatment plants to generate electricity and meet up to 50% of their electricity

demand through on-site generation of methane. However, if added above a certain quantity, it will inhibit methane gas production. Therefore, waste cooking oils from a restaurant has been chosen as a substrate. Long et al. (2012) also state that US EPA has estimated that more than 80% of municipalities that use anaerobic digestion to get rid of the solid wastes flare excess methane gas. However, if methane is trapped, it can be used to produce energy and replace fossil fuels.

This project also investigated the amount of methane produced if thickened waste activated sludge from the waste water treatment plant is co-digested with food waste and waste cooking oils from a restaurant. Literature has shown that much research has been done on anaerobic co-digestion, however, research on the combination of food waste, waste cooking oils and thickened waste activated sludge as substrates has not been done so far.

## 1.3 OBJECTIVES

The main aim of the project was to investigate methane production by anaerobic co-digestion of food waste, thickened waste activated sludge, and fats, oil & grease using Automatic Methane Potential Test System. The detailed objectives were:

- To investigate methane production if anaerobic digestion of food waste, waste cooking oil and thickened waste activated sludge was carried out individually
- To investigate methane production if different percentages of food waste, waste cooking oil and thickened waste activated sludge were co-digested anaerobically
- To investigate the stability of the digestion process at laboratory scale if high proportion of oil and grease is present in the feedstock

All the experiments were carried out in mesophilic temperature range (37ºC).

## 1.4 JUSTIFICATION

Anaerobic digestion of food waste, waste cooking oils and thickened waste activated sludge as co-substrates to produce methane has not been investigated. If methane production is in good quantity, this research would help the food industries to get rid of their food wastes and waste cooking oils, and this waste will help in production of methane which can be used as a fuel. Also, the waste water treatment plants may be able to get rid of the biological solid waste in a constructive way.

Producing methane using individual substrates helped in comparing the methane produced when all three substrates were used. Using different percentages of substrates in four combinations helped in investigating the optimum composition for co-digestion to produce methane.

The aim was to dispose the food waste, waste cooking oils and waste activated sludge and to obtain the highest yield of methane, which can be used as a fuel. Long et al. (2012) state that though enhanced biogas production during anaerobic co-digestion of fats, oils and grease has been often reported in the recent reports, not much discussion or research has been performed on its inhibitory effect or potential inhibition of methane production because of fats, oil and grease. Therefore, this project aimed to find out the optimum percentage of fats, oil and grease in the form of cooking oils, used as a substrate, with food waste and thickened waste activate sludge.

Baere L. (2006) state that setting up an anaerobic digestion plant involves high investment. Therefore, it would be helpful if the experiments are carried out at laboratory scale to find the optimum percentage of co-substrates and to analyse the methane production potential of substrates.

Anaerobic digestion has gained popularity in the last few years (Elsevier B.V. 2017). Figure 1 shows the number of documents published in each year for anaerobic co-digestion involving food waste. It is evident that there has been an increased interest in use of food waste as a co-substrate in the last ten years.

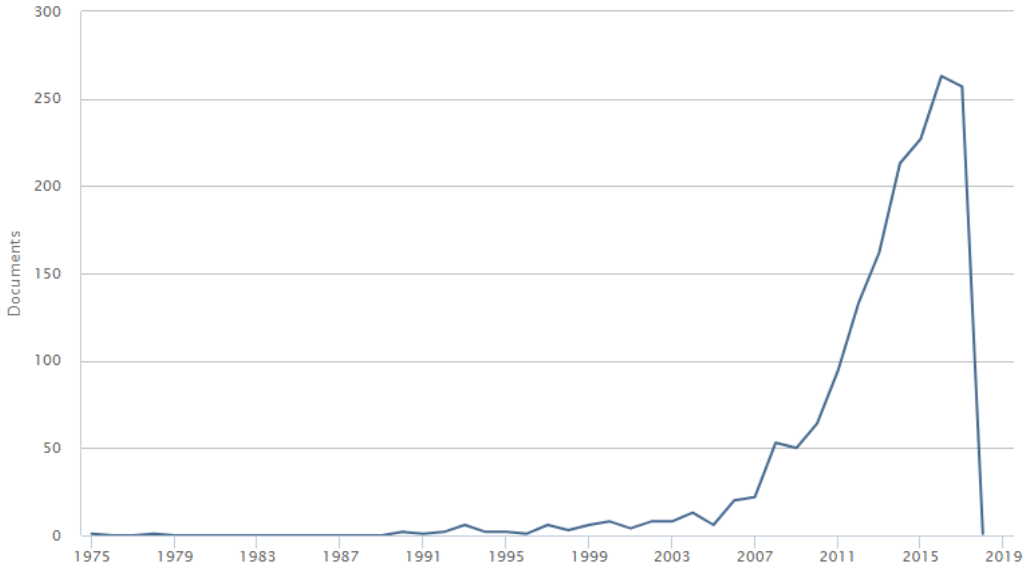


**Figure 1- Documents published by year of anaerobic co-digestion of food waste (Elsevier B.V. 2017)**

Figure 2 shows the number of documents published for anaerobic co-digestion of FOG. Approximately 34 papers were published in 2015 and 37 papers in 2016.

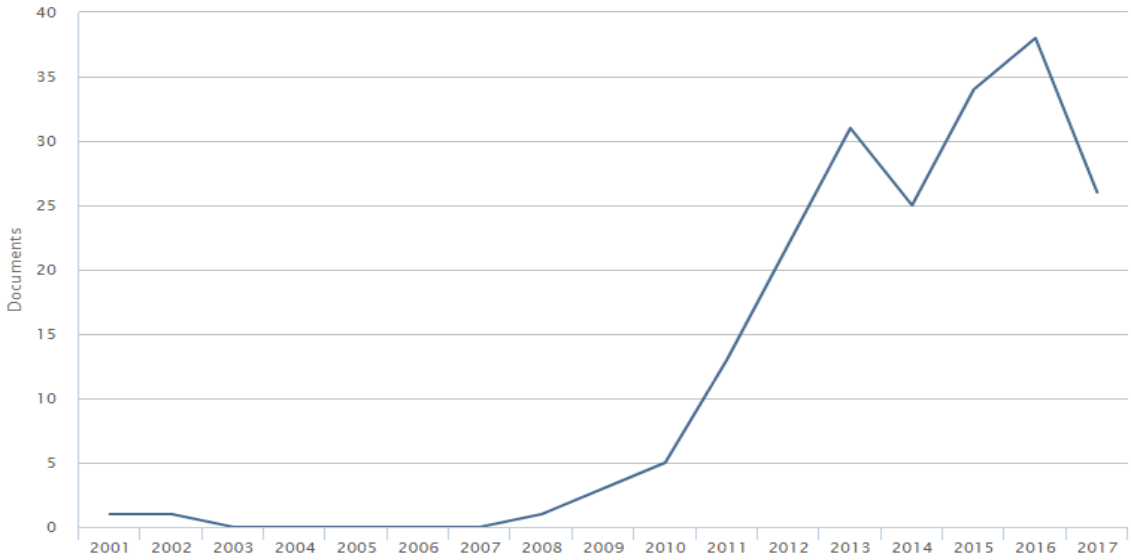


**Figure 2- Documents published by year of anaerobic co-digestion of FOG (Elsevier B.V. 2017)**

Figure 3 shows the number of documents published for anaerobic co-digestion of TWAS. The interest in TWAS as a co-substrate has increased in the last seven years.

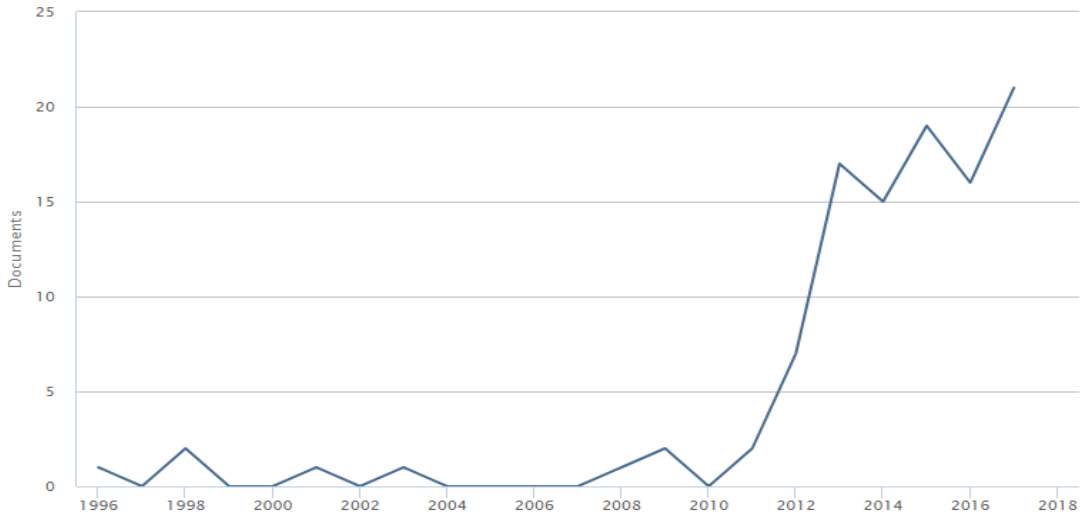


**Figure 3- Documents published by year of anaerobic co-digestion of TWAS (Elsevier B.V. 2017)**

It can be concluded that food waste has been a substrate of interest for anaerobic co-digestion. However, FOG and TWAS are not as popular as FW.

## 1.5 SCOPE

This study focused primarily on the production of methane using AMPTS from FW, FOG and TWAS. Experiments were conducted for single substrates as well as for all the three substrates together. Results obtained from single substrate digestion and co-digestion were compared with each other and with other studies. This study helped in investigating if co-digestion leads to more production of methane. Also, it helped in investigating if FW, FOG and TWAS could be used together efficiently as co-substrates.

# CHAPTER 2: BACKGROUND AND LITERATURE REVIEW

## 2.1 INTRODUCTION

In this chapter, anaerobic digestion will be discussed alongside the literature review conducted for this project. The first section introduces anaerobic digestion. Within this section, certain aspects of anaerobic digestion are discussed. In the second section, AMPTS is introduced. In the next section, literature review concerning anaerobic digestion is presented. To conclude this chapter, research gaps found during the literature review are presented.

## 2.2 INTRODUCTION TO ANAEROBIC DIGESTION

### 2.2.1 Methane as an alternative to fossil fuels

There is a growing interest towards the use of renewable resources of energy. As the non-renewable sources will not last long and because of the environmental concerns due to increasing levels of pollution and carbon dioxide emission level in the environment, there is a need to invest in renewable energy technology. Burning of fossil fuels poses a threat to environment. Therefore, there needs to be a replacement for fossil fuels. Sims et al. (2003) state that coal is the largest source for electricity generation (38%). 7700 million tons of carbon dioxide per year is released to the atmosphere by global electricity supply sector. This accounts for 37.5% of the total carbon emissions added per year. If use of fossil fuels is continued, 4000 Mt of C is expected to be the annual carbon emissions associated with electricity generation. Whiting and Azapagic (2014) state that global warming potential of energy generation from anaerobic digestion of waste is 50% lower than for fossil fuels.

### 2.2.2 Disposal of organic waste

Disposing the organic waste generated from various human activities is also a growing concern. Waste generated from food industries, including waste cooking oils, can be difficult to dispose. Kabouris et al. (2009) state that restaurants and food service providers and residences are major contributors of food waste. Food waste, especially, waste cooking oils may cause sewer problems by restricting the sewer flow and causing sewer overflows.

Chan and Schapper (2010) mention that in Australia, every year one person throws away 145kg of food. An illustration of this would be if a person buys five bags of groceries per week, he would end up disposing one bag of grocery. In total, 3.28 million tons of food waste is thrown away by Australian homes and businesses per year. Food waste comprises over 47% of the domestic waste stream and 70% of the commercial and industrial waste stream. In commercial and industrial sector, food organic waste makes up 50-60% of land-filled waste. Local governments are facing problems in disposing off this waste as it causes several problems including community health and safety standards, and visual and olfactory aesthetics of this waste. However, more important problem is when the food waste decomposes in the landfills, methane gas is produced. Methane gas causes twenty times more impact as a greenhouse gas than carbon dioxide (Larson and Ryan 2008). Methane production by one ton of food waste that is sent to landfills is equivalent to 750kg of carbon dioxide. The greenhouse impact caused by organic waste, including food waste, has been reported to be greater than 100,000 tons of carbon dioxide each year.

Zhang et al. (2014) state that waste is considered as one of the most promising energy sources for production of renewable energy. It is also stated that food waste is being increasingly produced by economic development and economic growth in both developing and developed countries. Figure 4 shows the amount of food waste in some countries.

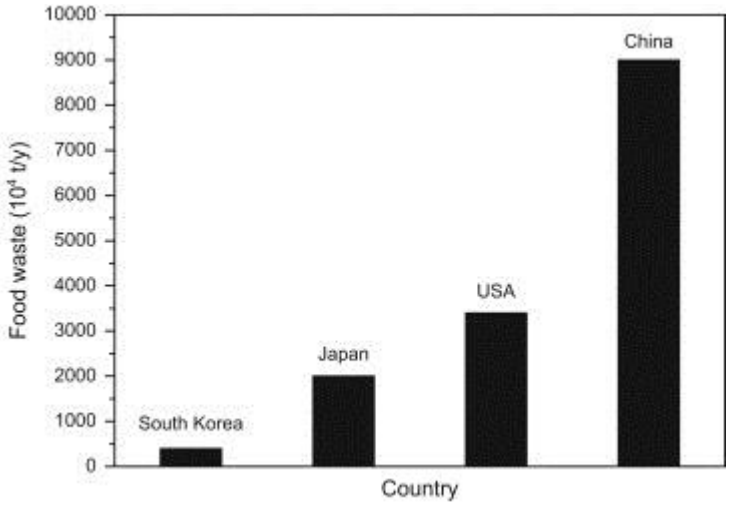


**Figure 4- Food waste in some countries (Zhang et al. 2014)**

### 2.2.3 Utilization of Thickened waste activated sludge

According to Wang et al. (2017), average annual production of excess sludge is 3 million wet tons in Australia, and 240 million wet tons in Europe, USA, and China combined. Landfill, agricultural use and incineration are still the common ways for sludge disposal. These methods incur very high costs, $30-$70 per wet ton in Australia. Therefore, anaerobic co-digestion is an effective way of utilizing this sludge for energy production. Excess sludge can be categorized as primary and secondary (waste activated) sludge. Waste activated sludge is composed mainly of bacteria growing on organic and inorganic substances, extracellular polymeric substances excreted by bacteria, recalcitrant organics formed during the decay of bacteria, and some inorganics from wastewater.

To deal with the growing environmental concern due to release of toxic compounds by burning fossil fuels and to deal with the growing organic waste disposal problem, which poses a threat to environment quality, anaerobic digestion of waste to generate methane gas is an appropriate solution.

### 2.2.4 Anaerobic digestion

Wu (2007) state that anaerobic digestion is a process in which microorganisms break down biodegradable material in the absence of oxygen. Various organic wastes can be treated by anaerobic digestion and bio-energy is recovered in the form of biogas, which contains mainly methane and carbon dioxide. This gas can be treated to recover methane which can be used as a source of energy. Co-digestion is the digestion of a homogenous mixture of two or more substrates simultaneously. Co-digestion provides a better nutrient balance which leads to better digester performance and higher methane yields. It also provides the benefit of providing with the required C/N ratio and a highly buffered system. Anaerobic co-digestion is carried out by several populations of bacteria. Whiting and Azapagic (2014) state that, typically, anaerobic digestion produces biogas, a mixture of 50-75% methane and remaining carbon dioxide. The biogas can be used to generate heat and/or electricity. Typical reaction for anaerobic digestion (Module: *Sludge treatment and disposal*):

$$\text{Organic matter} + \text{Combined Oxygen} \xrightarrow[\text{microbes}]{\text{Anaerobic}} \text{New cells} + \text{Energy for cells} + CH_4 + CO_2 + \text{Other end products}$$

Combined oxygen includes the radicals of carbonate, sulphate, nitrate and phosphate. Other end products are hydrogen sulphide, hydrogen and nitrogen.

Braber (1995) state that organic matter can be decomposed in two ways depending on the availability of oxygen. If the organic matter decomposes in the absence of oxygen (anaerobic digestion), a mixture of mainly methane and carbon dioxide is produced. If this decomposition takes place in the presence of air (aerobic digestion), then no biogas is produced. Moreover, waste such as food waste, is too wet and it lacks structure to undergo aerobic digestion. Also, aerobic generation is an energy intensive process. Anaerobic

digestion generates energy, and is gaining popularity. Tauseef et al. (2013) have compared the anaerobic digestion and aerobic digestion as mentioned in the Table 1.

**Table 1- Comparison of anaerobic and aerobic digestion (Tauseef et al. 2013)**

| **Aspect** | **Anaerobic digestion** | **Aerobic digestion** |
|---|---|---|
| Energy requirements | Low | High |
| Extent of loading possible | High to very low (Typical organic loading rate – 3.2-32 kg COD/$m^3$ day) | Moderate to very low (Typical organic loading rate – 0.5-3.2kg COD/$m^3$ day) |
| Degree of treatment | High (>90%) | (>95%) |
| Sludge production | Very low | Much higher |
| Process stability (to toxic compounds and load changes) | Good | Good |
| Startup time | 2-4 weeks | 2-4 weeks |
| Nutrient requirements | A fifth or lesser than aerobic processes | Higher than 5 times for certain industrial wastes |
| Odour problems | Low, as the systems are air-tight | Low, despite systems being largely open |
| Energy production | Yes | No |
| Nutrient recovery | Possible | Not possible |
| Effluent quality | contains higher suspended solids and ammoniacal nitrogen | |

Braber (1995) also state that anaerobic digestion occurs in nature by itself where the right typical conditions are present, like, bottom of the lakes, landfills etc. However, when this

process is carried in plant, the conditions such as temperature, humidity, microbial activity, and waste properties, are controlled. This leads to a stimulated and accelerated process. Anaerobic digestion is carried out by a consortium of four different types of microorganisms: hydrolytic, fermentative, acetogenic, and methanogenic. Typical composition of biogas is shown in the Table 2.

**Table 2- Biogas properties (Braber 1995)**

| | |
|---|---|
| Energy content | 20-25 $MJ/m^3$ |
| Methane (volume %) | 55-70 |
| Carbon dioxide (volume %) | 30-45 |
| Hydrogen sulphide | 200-4000ppm |

Theoretically, 1kg COD can be converted to 0.35 $m^3$ of methane (Lin et al. 1997; Module: Sludge treatment and disposal). 3.28 million tons of food is thrown as waste every year in Australia (Schapper and Chan 2010). If the energy from this waste is harnessed it would lead to generation of electricity. If 95% of the total food waste is the volatile solids (organic wastes), then 3.116 million tons of the organic food waste can be used to generate energy. This quantity of food waste will generate $1.09*10^9$ $m^3$ methane/year. If 60% of the biogas is methane, then $1.1817*10^9$ $m^3$ biogas/year can be produced. If it is assumed that 22$MJ/m^3$ of energy is released (Braber 1995), then it would lead to $1.11*10^{10}$ kWh of energy. Electrical energy which can be generated from this energy (if it is assumed that the efficiency of the generator is 30%), then $3.332*10^9$ kWh of electricity can be harnessed from the food waste. This energy can be used to provide electricity to about 2 million people.

Zhang et al. (2014) state that investigations were conducted for possible treatment technologies for organic waste in London. The three technologies used were- i) landfill with electricity production, ii) incineration with steam recovery for combined heat and power, and iii) anaerobic digestion with energy recovery. Life cycle inventory data showed that the best treatment for organic waste is anaerobic digestion.

Braber (1995) also state that anaerobic digestion is a net energy production process (150-250 kWh per ton of input waste) but its commercialization is not yet fully demonstrated. On the other hand, composting is an energy consuming process (around 30-35 kWh is consumed per ton of waste input). Vandevivere et al. (2003) also state that anaerobic digestion which involves hydrolysis, methanogenesis and biogas valorization produces electricity (150-300$kWh_{elec}$/ton of waste) or heat (250-500 $kWh_{heat}$/ton of waste). However, there are successful plants running currently using anaerobic digestion, which offers some advantages as well as disadvantages, which could be overcome with time and research, as mentioned in the Table 3.

**Table 3- Advantages and Disadvantages of Anaerobic digestion (Braber 1995)**

| Advantages | Disadvantages |
|---|---|
| • Net production of energy<br>• Reduced carbon dioxide emissions, by displacing fossil fuels<br>• Potential to deal with the wet fraction of waste, such as food waste, which is less amenable to incineration<br>• Potential to treat the waste in countries where landfilling of waste could be banned<br>• Reduction of odour<br>• Lower land requirement in comparison to other techniques, such as aerobic composting<br>• The residue can be sold as a soil amendment or combusted to recover more energy<br>• Potential for co-disposal of different types of organic wastes<br>• Environmentally benign waste treatment | • More expensive than composting in many cases<br>• It is a novel application; Information on economic and practical issues is not widely spread<br>• Additional pre-treatment may be required.<br>• More susceptible to upsets due to toxic substances<br>• pH adjustment may be required |

Gujer and Zehnder (1983) have identified six different conversion processes which take place in the degradation of organic matter to methane.

- Hydrolysis or liquefaction- High molecular insoluble organic polymers such as carbohydrates, lipids and proteins are hydrolyzed. They are converted to soluble fragments (monomers) such as sugars, amino acids, long chain fatty acids by enzymes.
- Acidogenesis- Fermentation or acidogenesis of amino acids and sugars by which organic monomers are converted to acetic, propionic and butyric acids, hydrogen, carbon dioxide, ethanol, lactic acid etc. This process is carried out by acidogenic bacteria.
- Acetogenesis- Long chain fatty acids and alcohols are converted to acetic acid, hydrogen, carbon dioxide during anaerobic oxidation by obligate hydrogen producing acetogenic bacteria.
- Intermediate products such as volatile acids (except acetate) are anaerobically oxidized.
- Methanogenesis- Conversion of acetate to methane by acetolastic methane fermentation. This process is carried out by archaea.
- Methanogenesis- Conversion of hydrogen to methane

The processes are depicted in the figures 5 and 6 below:

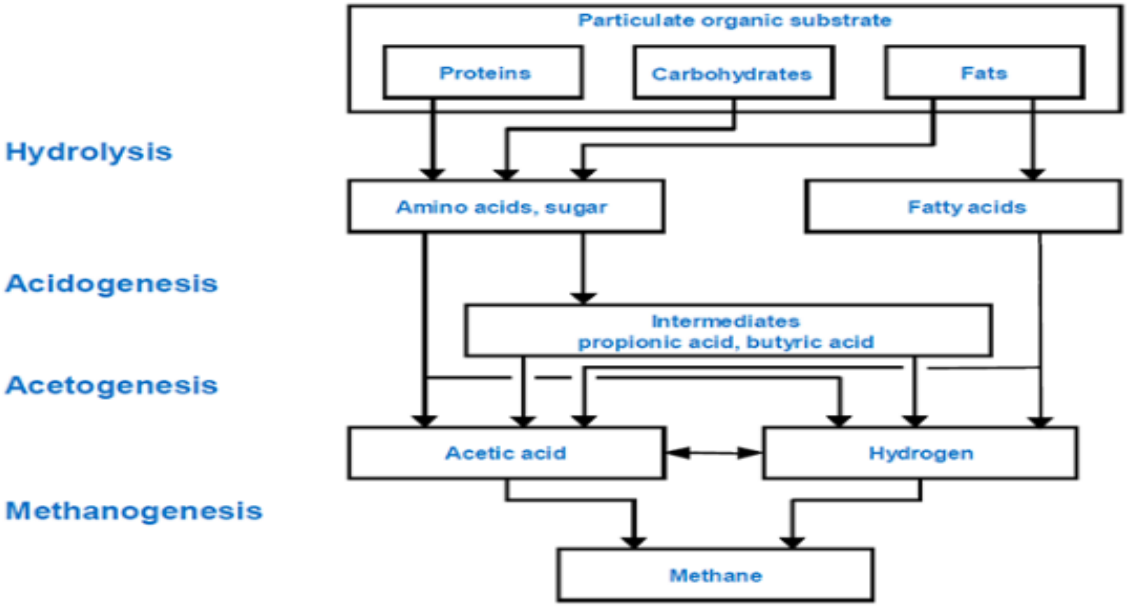


**Figure 5- Degradation steps of anaerobic digestion process (Emmanuel Serna 2009)**

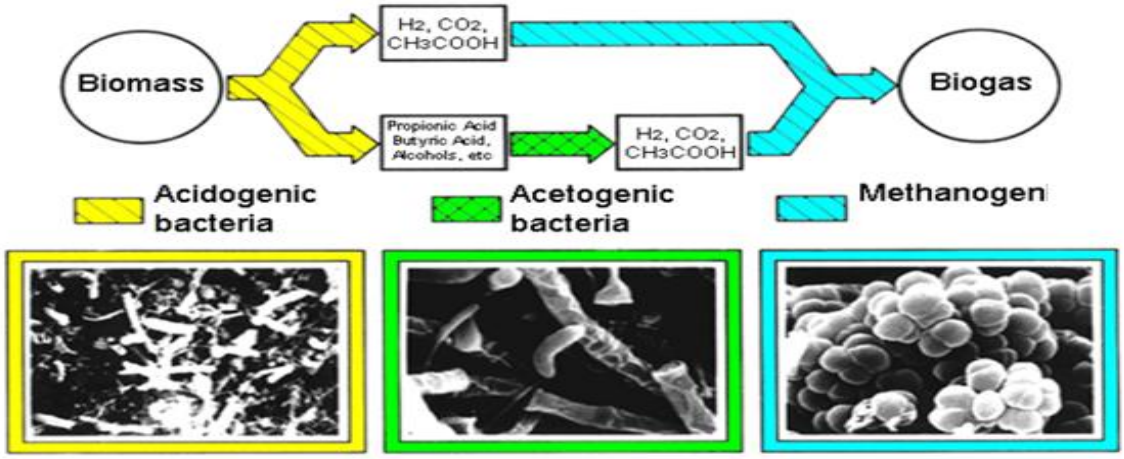


**Figure 6- Schematic representation of the course of anaerobic methane generation from complex organic substances showing scanning electron micrographs of individual microbes involved (Emmanuel Serna 2009)**

Tauseef et al. (2013) mention that bacterial cells try to achieve maximum of cell growth for the least amount of energy expended. In anaerobic digestion, no molecular oxygen is present and the environment is reducing in nature. The oxidation-reduction potential values are approximately -490 to -550 mV. Carbon atoms associated with some of the organics become

electron acceptors and are reduced under anaerobic conditions, while other organics are oxidized to carbon dioxide and volatile acids. This reaction results in end products that still contain enormous amounts of energy (i.e. potential to accept electrons) in the form of methane. Cell reproduction in anaerobic conditions is not very high. It is also stated that the methanogenic bacteria are pH sensitive. The bicarbonate is often used to maintain an optimum value of pH in the digester.

## ***INHIBITION OF ANAEROBOC DIGESTION PROCESS***

Chen et al. (2008) state that there are various inhibitory substances which lead to failure of anaerobic digestion. Inhibition is usually indicated by accumulation of organic acids and decrease in the methane gas production at steady state rate. Commonly present inhibitors are ammonia, sulphide, organics, light metal ions, and heavy metals. Co-digestion of wastes and adaptation of microorganisms to the inhibitory substances can help in improving the digestion efficiency.

Ammonia is one of the common inhibition cause. It is produced by the biological degradation of the nitrogenous matter. Main forms of inorganic ammonia nitrogen in aqueous solution are ammonium ions and free ammonia. Of all the anaerobic microorganisms, Methanogens are the least tolerant of ammonia whereas Acidogenic bacteria are hardly affected by ammonia. Air-stripping and chemical precipitation are the commonly used methods to remove ammonia from the substrate.

In anaerobic digestion, sulphide is another cause of inhibition. It is formed when the sulphate is reduced to sulphide by the sulphate reducing bacteria. Sulphide results in primary and secondary inhibition. Primary inhibition occurs due to the competition of common organic and inorganic substrates from sulphate-reducing bacteria. Secondary inhibition occurs due

to the toxicity of sulphide to various bacteria groups. Sulphide inhibiton can be controlled by diluting the wastewater rich in sulphates and by incorporating sulphide removal techniques such as stripping, coagulation, oxidation and precipitation.

Light metals such as sodium, potassium, magnesium, calcium and aluminum also result in inhibition. These metals are present in the influent of the anaerobic digesters. Salts of these metals are inhibitory as they cause bacterial cells to dehydrate due to osmotic pressure. Moderate amounts of these metals stimulate microbial growth whereas in massive quantities, they are toxic. Heavy metals such as cobalt, copper, zinc, chromium, cadmium and nickel can accumulate in the anaerobic digester to toxic concentrations. The toxic effect is due to the disruption of enzyme function and structure when the metals are bonded with thiol and other groups on protein molecules. Inhibition by heavy metals is very common. Precipitation, sorption and chelation by organic and inorganic ligands are some common methods of coping with the heavy metal toxicity.

Inhibition by organics such as long chain fatty acids, alkyl benzenes, halogenated benzenes, alkanes, aldehydes, ether, nitrophenols, carboxylic acids is also common.

## 2.3 INTRODUCTION TO AMPTS

Stromberg et al. (2015) state that AMPTS is a recent development which allows automatic and reliable gas measurements with high resolution and makes an approach based on real-time prediction with mathematical models feasible. AMPTS is a standardized laboratory set-up designed for automatic bio-methane potential testing of any biodegradable material. It consists of pre-calibrated flow cells in which gas is measured through water displacement. It gives a signal for every 10mL of produced gas. The gas volume is normalized to 0˚C, 1 atm and dry gas conditions at each measuring point by temperature and pressure sensors.

Bioprocess Control AB (2017) has mentioned that AMPTS can be used to measure ultra-low bio-methane flows at laboratory scale. Sodium hydroxide (reagent grade 97%, Sigma–Aldrich) and thymolphthalein pH indicator (dye content 95%, Sigma–Aldrich) are used to prepare 3M alkaline solution for the absorption of carbon dioxide. Anaerobic conditions during the preparation phase are generated by passing nitrogen gas through the system. Temperature control is up to 95 °C.

It consists of 3 components (Figure 7):

- Sample incubation unit
- Carbon dioxide Fixing unit
- Flow cell array unit

**Figure 7- AMPTS (Bioprocess Control AB 2017)**

Some of the advantages AMPTS offers are (Bioprocess Control AB, 2017):

- Automatic analytical procedure reducing work load
- User-friendly interface for experimental set-up and real-time data display
- Real-time gas flow and volume normalization
- On-line and real-time data logging of total bio-methane production and flow rate

- Low and easy maintenance

## 2.4 BACKGROUND OF ANAEROBIC CO-DIGESTION

Study of the literature investigating the anaerobic digestion involving single and multiple substrates was done and the main takeaways are documented below.

A review by Mata-Alvarez et al. (2014) reported that in between 2010 and 2013, the most popular main substrates were animal manure (54%), sewage sludge (22%), and organic fraction of municipal solid waste (11%). The most popular co-substrates were industrial waste (41%), agricultural waste (41%), and municipal waste (20%).

Grosser et al. (2017) investigated the anaerobic co-digestion of sewage sludge with grease trap sludge and municipal solid waste. The study was performed in semi-continuous mode at mesophilic conditions. Total solids content in grease trap waste (GTW) was 60.48%, in sewage sludge was 2.4-3.93% and in the organic fraction of municipal solid waste (OFMSW) was 17.5-20.3%. Volatile solids/Total solids (VS/TS) ratio in GTW, sewage sludge and OFMSW were 0.95, 0.96 and 0.71-0.78 respectively. COD in the sewage sludge was 31240-52773mg/L. Carbon/Nitrogen ratio of sewage sludge and Organic fraction of Municipal solid waste were 9.05-21.5 and 29 respectively. Organic loading range for was in the range of 1.5 to 2.5 VS/Ld. It was found that the organic loading rate for fats, oils and grease (FOG) was not clear enough. Safe co-digestion performance and high biogas productions have been recorded for organic loading rate for FOG up to 0.8 kg VSFOG $m^{-3}$ $d^{-1}$. Average methane yield of sewage sludge (which has been calculated from a period of 27-70 days) was 300L/kg VSS. Co-digestion process produced more methane than obtained when sewage sludge was anaerobically digested alone. Co-digestion of sewage sludge and grease trap sludge at a ratio of 30, increased the methane production by 52% (i.e. from 300

to 456 $m^3$/mg $VS_{added}$). When OFMSW was used as a co-substrate with sewage sludge, the average methane production increased up to 82% (i.e. from 300 to 547$m^3$/mg $VS_{added}$). The additional methane production is mainly due to increase of fat content in the feedstock, which is characterized by high methane potential, much higher than proteins and carbohydrates. C/N ratio improved after co-digesting which also led to increase in the methane production.

Xie et al. (2017) conducted sets of experiments to study the anaerobic digestion. Food waste, paper pulp reject and primary sludge were anaerobically digested individually. Co-digestion of combination of food waste (FW) and primary sludge (PS) and combination of paper pulp reject (PPR) and sewage sludge (PS) was also performed. It was found that the process performance enhanced when co-digestion was performed. Cumulative methane production from co-digestion of food waste and primary sludge and paper pulp reject with food waste was more than the production from mono-digestion. Specific methane production for co-digestion of PS and FW was 799mL/g VS added and in case of PS and PPR was 368 mL/g VS added. Specific methane production for mono-digestion of PS, FW and PPR were 159mL/g VS, 652mL/g VS, and 157mL/g VS respectively. Synergistic effect was obtained in lag phase. When co-digestion was performed the lag phase got reduced in both cases. Lag phase for mono-digestion of PS, FW and PPR were 0.91 day, 0.46 day, and 0.89 day whereas in co-digestion of PS with FW was 0.25 day and in co-digestion of PS with PPR was 0.54 day. Figure 8 depicts the methane production from mono-digestion and co-digestion.

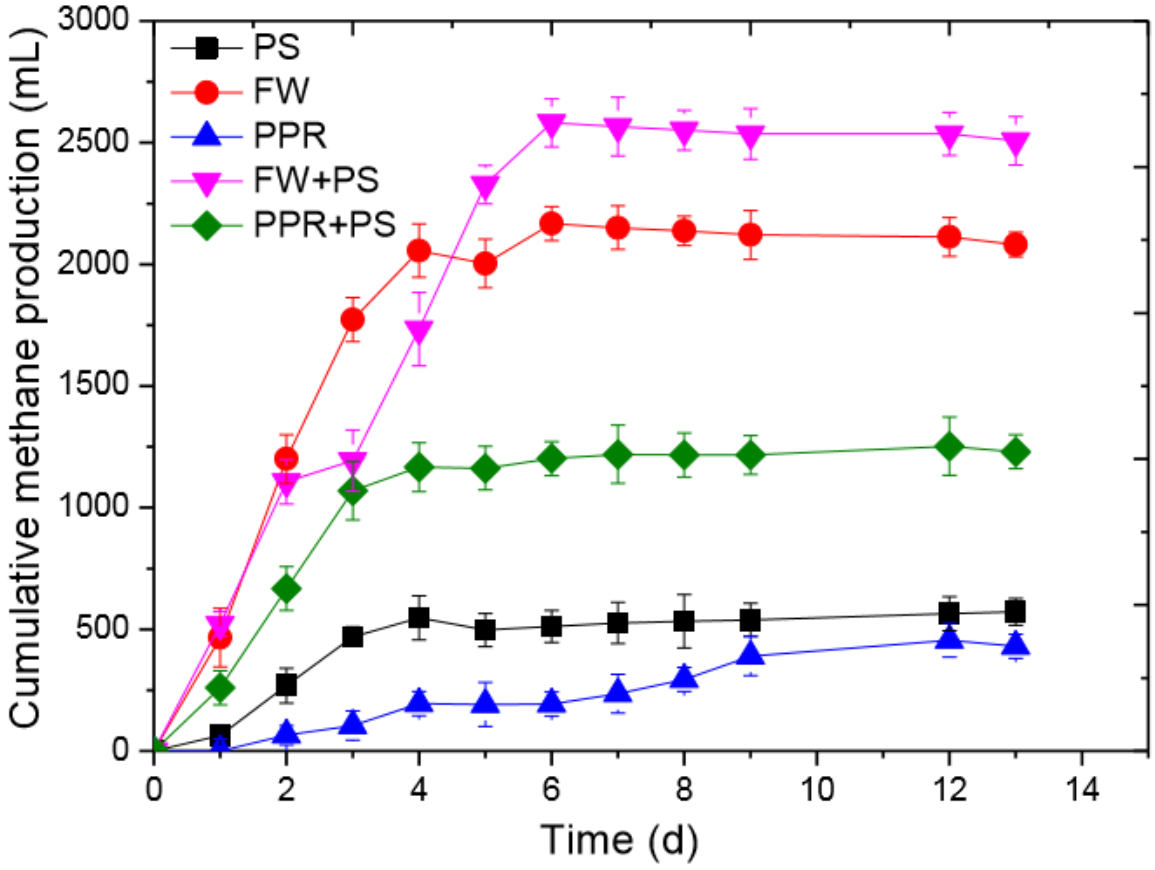


**Figure 8-Cumulative methane production from mono-digestion and co-digestion of Primary sludge with organic wastes (Food waste and paper pulp reject) (Xie et al. 2017)**

Alqaralleh et al. (2016) studied the anaerobic co-digestion of thickened waste activated sludge (TWAS) with fat, oil and grease (FOG) and evaluated the methane production. Volatile solids (VS) in TWAS, FOG and inoculum were 34.5g/Kg, 282.8g/Kg and 14.7g/Kg respectively. Experiments were performed using different percentages of FOG and it was found that with the increase of FOG as substrate up to a specific amount significantly increased the methane production. The control sample, which contained the inoculum and TWAS (0% FOG) produced 316.4 ml methane. Addition of 20%, 40% and 60% (based on TVS) FOG to the co-digestion mixture increased the methane production to 427ml, 451ml, and 491ml respectively. This represents 35.2%, 42.6% and 55.4% increase in methane production in comparison to the control. However, addition of 80% FOG to the co-digestion

mixture reduced the methane production to 102ml, which is less than the methane production for the control. Therefore, FOG has an inhibitory effect at 80% composition. Figure 9 depicts the methane production for different percentages of FOG in the anaerobic mixture.

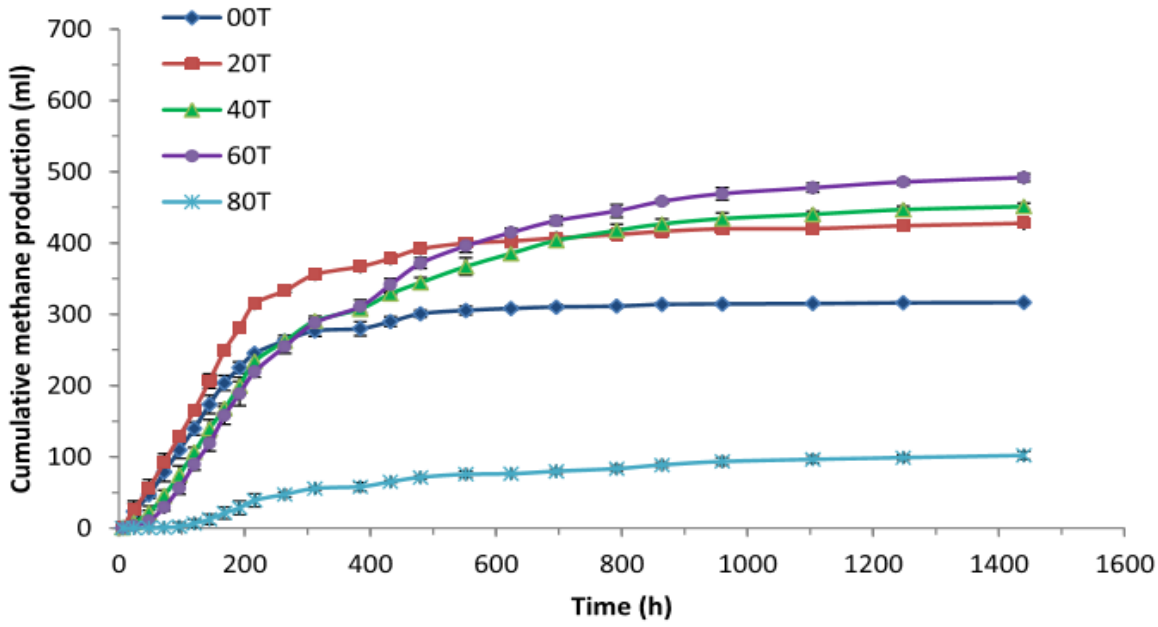


**Figure 9- Cumulative methane production for different percentages of FOG in co-digestion mixture (Alqaralleh et al. 2016)**

This indicates that high concentration of FOG leads to high concentrations of long chain fatty acids, which have toxicity effect on anaerobic micro-organisms, especially the methanogens. This effect can be explained by long chain fatty acids adsorption onto methanogen cells which cause damages to the cell membranes and it becomes difficult for them to absorb the nutrients. However, the exact nature and mechanism of the inhibitory effect of long chain fatty acids is not clear enough.

Li et al. (2011) evaluated the biogas production from municipal fat, oil and grease (FOG) and kitchen waste (KW) in anaerobic co-digestions. Two sets of experiments were performed. In set 1 experiment, FOG and KW were investigated as single substrates when

added to the inoculum. In the second set of experiments, FOG and KW were tested as co-substrates when added to waste activated sludge alternatively. Co-substrates were added to the prepared waste activated sludge and inoculum mixture with optimized substrate/inoculum (S/I) ratios based on the results obtained from single substrate digestion experiments. From the experimental results, it was obtained that when KW was digested as a single substrate, 6g KW with S/I ratio of 1.03, a higher methane production was observed than other cases using KW at different S/I ratios. Also, for digestion using FOG as single substrate, higher cumulative methane production was obtained in case of 0.35g FOG with substrate/inoculum (S/I) ratio of 0.5 than other cases with different S/I ratios. In case of KW mono-digestion, higher methane production was achieved than the blank except the case when 12g KW was added. On the other hand, in case of FOG mono-digestion, higher cumulative methane yields were obtained than the blanks just in case of 0.35g FOG. Also, for the digestions using more than 1.4g of FOG with S/I ratios higher than 2, negligible methane production was obtained. Initial COD and VS concentrations were higher in FOG than KW, and as a result, the anaerobic consortium was much more sensitive to FOG than KW. Therefore, suitable S/I ratios are important for methane production for pilot and full-scale reactors. Figure 10 shows the methane production (ml) per g/VS in case of mono-digestion of FW and FOG as well as their co-digestions alternatively with waste activated sludge.

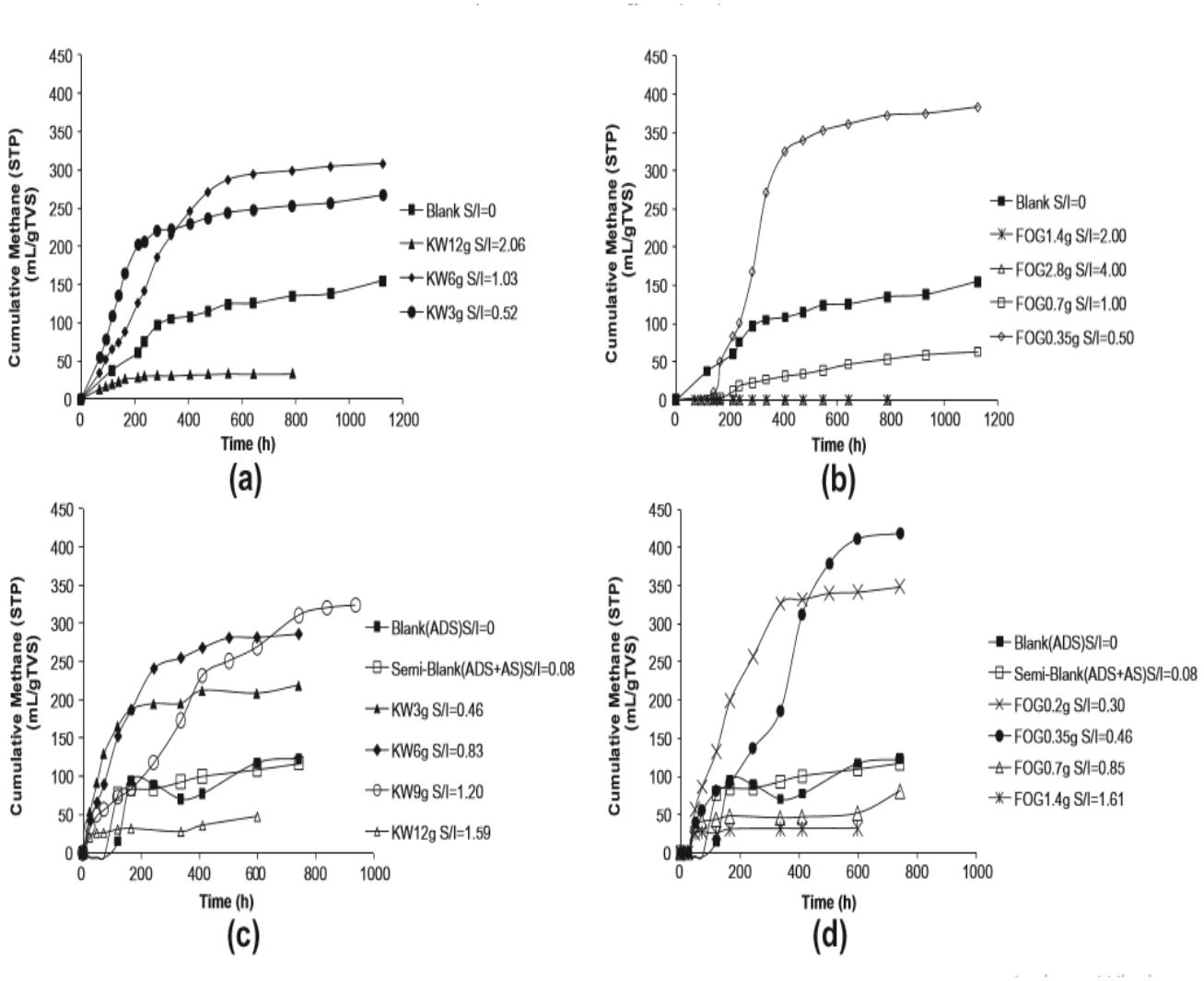


**Figure 10- Cumulative methane production from single substrate using (a) Kitchen waste (b) Fat, oil and grease as single substrates as well as co-digestion experiment using (c) KW and (d) FOG as co-substrates of waste activated sludge (Li et al. 2011)**

## 2.5 RESEARCH GAPS

After undertaking the literature review, it was found that there is excessive food waste, waste cooking oils and waste activated sludge being produced around the world. Use of anaerobic digestion will help in disposing of the waste as well as harnessing energy from it. Biogas can be used to substitute fossil fuels which are causing carbon dioxide emissions which lead

to global warming. Also, it was found that though there are some disadvantages of anaerobic digestion, it was a better way of disposing waste than landfilling and aerobic digestion.

It was also found that good research has emerged on anaerobic digestion in recent years. Studies using different substrates such as agricultural, municipal and industrial wastes have been performed and it was found that they can be digested successfully and efficiently together (Grosser et al. 2017). Studies have also been conducted on FOG with activated sludge (Kabouris et al. 2009; Luostarinen et al. 2009; Razaviarani et al. 2013) as well as food waste with activated sludge (Li et al. 2011), and it was found that co-digestion enhances the methane production. However, there is no information regarding co-digestion of food waste, waste cooking oils and thickened waste activated sludge in a single study as co-substrates. Therefore, the project aimed to fill the existing gaps.

# CHAPTER 3: METHODOLOGY

## 3.1 INTRODUCTION

This chapter documents the methodology for the project. Collection and characterisation of food waste, thickened waste activated sludge, waste cooking oil and inoculum, characterisation of inoculum and substrates, AMPTS and the set of experiments will be discussed.

## 3.2 COLLECTION OF FOOD WASTE, TWAS, FOG & INOCULUM

The food waste and waste cooking oils used in the experiments were obtained from the University of Southern Queensland Refectory, Toowoomba, Australia. The food waste comprised of a mixture of chips, bacon, fruits and their peals, and bread. This food waste was grinded to form a slurry. FOG used in this study is mainly Canola oil.

Thickened waste activated sludge was obtained from the Wetalla Wastewater Treatment plant in Toowoomba. Figure 11 shows the thickened waste activated sludge in Wetalla treatment plant.

**Figure 11- Thickened Waste activated sludge at Wetalla Wastewater Treatment plant**

The inoculum was obtained from the pond at a piggery farm located in Lockyer Valley in Queensland, Australia. The inoculum is important for enabling the digestion process. Figure 12 shows the pond from where the inoculum was obtained.

**Figure 12- Pond at the piggery farm**

## 3.3 CHARACTERISATION OF FOOD WASTE, TWAS & FOG

Characterisation results helped in analysing the final methane production results obtained from the AMPTS experiments. Characterisation of food waste, thickened waste activated sludge and fat, oil and grease was performed to measure the following:

### 3.3.1 Total solids

Total solids test was performed for measuring total solids in food waste, waste cooking oil, thickened waste activated sludge, and inoculum. Three samples of each were placed in the crucibles and placed at 105℃ in an oven over-night. Samples with the crucibles were weighed before and after heating in the oven. There was a difference in the weight which was due to the lost moisture. Weights of the crucibles were reduced from the final readings which gave the measure of total solids. Figure 13 shows the crucibles with substrates and inoculum prior to placing them in the oven.

**Figure 13- Food waste, thickened waste activated sludge, Inoculum, waste cooking oil (from left) before heating in the oven at 150℃**

### 3.3.2 Total volatile solids

Total volatile solids analysis was also performed for food waste, thickened waste activated sludge, inoculum, and waste cooking oil. After measuring the total solids in the first analysis, the crucibles were kept in a furnace and heated at 550℃ for an hour. Crucibles with the ash were weighed. Weights of the crucibles were subtracted from the final reading to get the weights of ash in each of them. Difference between the weights of total solids was subtracted from the weight of ash to obtain the weight of volatile solids. Figure 14 shows the crucibles with substrates and inoculum after they were taken out from the furnace.

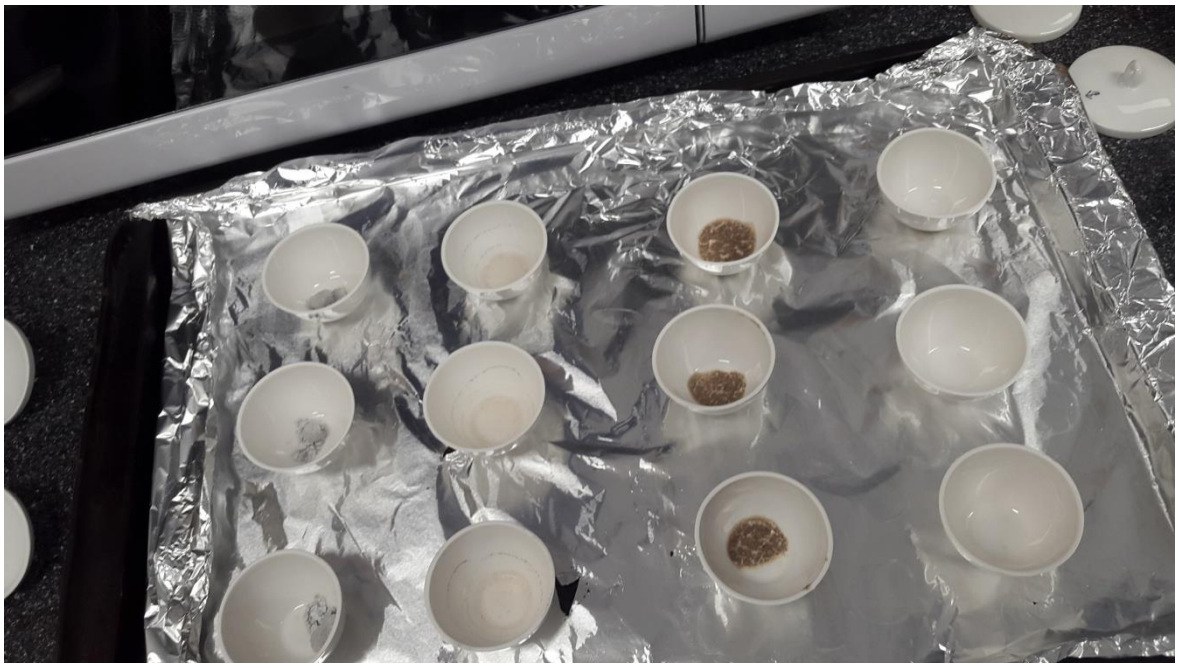

**Figure 14- Ash form of food waste, thickened waste activated sludge, Inoculum, waste cooking oil (from left) after heating in the furnace at 550℃**

### 3.3.3 Chemical Oxygen Demand

Chemical oxygen demand was analysed for food waste, TWAS and inoculum.

*COD for food waste*

Food waste was in the form of slurry. Therefore, it had to be diluted and filtered to make it compatible for measuring COD. The following steps were performed to measure the COD:

- 1g of food waste was diluted 25 times by adding 24ml distilled water.
- It was filtered using 5μm pore size filter.
- 2ml of the diluted solution was added to the potassium dichromate solution in the tube 1.
- 1ml of diluted solution was added to potassium dichromate solution in tube 2. 1ml of distilled water was added to dilute it to 50 times.
- COD was measured for both the samples using spectrophotometer.

*COD for inoculum*

Inoculum was too thick to conduct COD. Therefore, it had to be diluted to 50 times by adding 49ml of distilled water to 1ml of inoculum.

- 1ml of the diluted solution was added to the potassium dichromate solution in the tube 1. It was further diluted by adding 1ml of distilled water. Therefore, the dilution factor in this case was 100.
- 0.5ml of this diluted solution was added to potassium dichromate solution in tube 2. 1.5ml of distilled water was added to dilute it further. The dilution factor in this case was 150.
- COD was measured for both the samples using spectrophotometer.

*COD for thickened waste activated sludge:*

1ml of the sludge was added to the potassium dichromate solution. 1ml of distilled water was then added to dilute it 50 times. A similar sample was prepared in tube 2 to create a duplicate of tube 1. COD was measured for both the samples using spectrophotometer.

### 3.3.4 Total Organic Carbon

Total organic carbon analysis was performed for food waste and thickened waste activated sludge.

*TOC for unfiltered and hydrolysed food waste*

Food waste was in the form of slurry and therefore, not suitable for conducting the TOC analyses in the analyser directly. The particulate matter in the food waste was hydrolysed to soluble matter to get all the organic carbon as soluble. However, addition of sulphuric acid reduced the pH of the solution to 0.3. TOC analyser is not suitable to take the reading if pH is lower than 2.5. Therefore, sodium hydroxide was added to the food waste and sulphuric acid solution to neutralize the pH and make it close to 3. Following procedure was followed to perform the analysis:

- 98ml distilled water was added to 1g food waste.
- 1ml sulphuric acid was added to it for the hydrolysing the particulate matter.
- It was blended using a magnetic stirrer food waste dissolved.
- 10 pellets of sodium hydroxide were added to 10ml distilled water.
- Sodium hydroxide solution was added to the food waste solution while it was being stirred.
- Addition of this NaOH solution further increased the dilution.
- pH of this solution was monitored after adding each drop of NaOH solution.

- NaOH solution was added until the pH reached 2.7.
- Conductivity of the prepared solution was measured. The reading was 24.4mSiemens. However, the conductivity must be less than 2 mSiemens.
- To lower the conductivity, the solution was diluted. 0.5ml of this solution was added to 19.5ml of distilled water (dilution factor of 40) and the conductivity got reduced to 1.7 mSiemens.
- The overall dilution factor for the solution was 6000.
- A duplicate sample was prepared in the same procedure.
- Both the samples were placed in the TOC analyser to measure the total organic carbon.

*TOC for filtered food waste*

Two samples of filtered food waste were prepared for measuring the total organic carbon and comparing the results with the results obtained for unfiltered and hydrolysed food waste. 1g of food waste was added to 99ml distilled water and the solution was filtered using glass fiber filter paper. The tube was filled with this diluted solution and its conductivity was measured. It was 0.3mSiemens and hence, was suitable for the TOC analyser. A duplicate was prepared in the same way. Both the samples were placed in the TOC analyser. Overall dilution factor was 100.

*TOC for thickened waste activated sludge*

Two samples of filtered thickened waste activate sludge were prepared for measuring the total organic carbon. 1g of TWAS was added to 99ml distilled water and the solution was filtered using glass fiber filter paper. The tube was filled with this diluted solution and its conductivity was measured. It was 48.92 μSiemens and hence, was suitable for the TOC

analyser. A duplicate was prepared in the same way. Both the samples were placed in the TOC analyser. Overall dilution factor was 100.

### 3.3.5 Nitrogen

Nitrogen content was measured for food waste and thickened waste activated sludge.

*Nitrogen content in unfiltered and hydrolysed food waste*

The solution prepared for analysis of total organic carbon was used to measure the nitrogen content. However, the dilution factor in this case was 110 (as 19.5ml distilled water was not added in this case to reduce conductivity). Two samples of this solution were placed in the nitrogen content analyser.

*Nitrogen content in filtered food waste*

The solution prepared for measuring total organic carbon in filtered food waste was used to measure the nitrogen content. The same dilution factor of 100 was used.

*Nitrogen content in thickened waste activated sludge*

For measuring the nitrogen, the same solution was used that was prepared for measuring the total organic carbon. Two samples were placed in the nitrogen content analyser. Dilution factor for thickened waste activated sludge was 100.

## 3.4 PREPARATION OF BIO-MEDIUM

The bio-medium is essential for anaerobic digestion as it provides the micro-nutrients to the microbes which are responsible for the digestion process. Zhang et al. (2014) state that metal elements including light metal ions such as sodium, potassium, magnesium, calcium and aluminium as well as heavy metal ions such as chromium, copper, cobalt, nickel, zinc are

essential for anaerobic bacteria as these cations play a crucial role in enzyme synthesis and maintain enzyme activities. However, if these metal ions are present in high concentration they would cause inhibition. 6 Litres of bio-medium was prepared for both sets of experiments. The bio-medium for the experiments was prepared using the chemicals mentioned in the Table 4. After adding all the nutrients in distilled water, it was stirred using the magnetic stirrer. Figure 15 shows the prepared bio-medium. There were some risks involved while handling these chemicals. All the risks involved for these chemicals and substrates are mentioned in Appendix B.

**Table 4- Preparation of bio-medium (Owens et al. 1979)**

| Chemical | Final conc. Required in medium (mg/L) | Concentration (mg/L) for 6L of BM |
|---|---|---|
| Dipotassium phosphate | 653 | 3918 |
| Sodium Phosphate, Monobasic, Anhydrous | 149.96 | 899.77 |
| Resazurin | 0.5 | 3 |
| *Trace Elements solution:* | | |
| Ferric Chloride, Hexahydrate | 2.36 | 14.17 |
| Manganese Chloride | 0.64 | 3.82 |
| Cobalt Chloride, Hexahydrate | 0.19 | 1.14 |
| Zinc Chloride | 0.07 | 0.42 |
| Copper(II) Chloride | 0.14 | 0.85 |
| Sodium Molybdate | 0.03 | 0.22 |
| Nickel Chloride, Hexahydrate + 1 ml HCl | 0.02 | 0.14 |
| Ammonium Carbonate | 443.9 | 2663.4 |
| Sodium Bicarbonate | 3730 | 22380 |
| Sodium sulphide | 0.24 | 1.44 |
| Calcium chloride, dry | 83.04 | 498.25 |
| Magnesium Chloride, Hexahydrate | 122.76 | 736.53 |

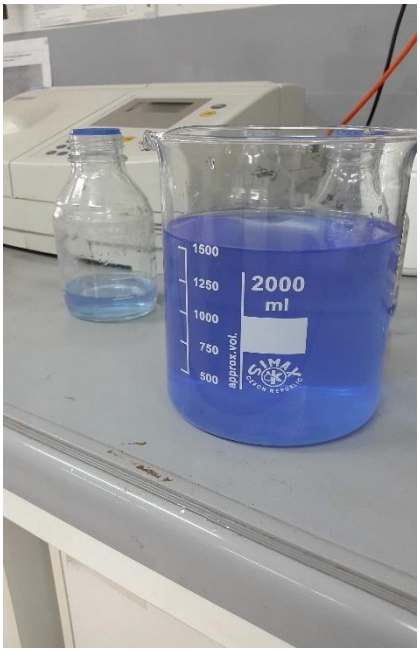


**Figure 15- Bio-medium**

## 3.5 SETS OF EXPERIMENTS USING AMPTS

Bioprocess Control AB (2017) mentioned that AMPTS can be used to measure ultra-low biomethane flows at laboratory scale. The analyzing principles used in conventional methane potential tests are used in AMPTS. However, AMPTS offers the benefit of automatically measuring the gas volumes as well as logging data during long experimental periods. AMPTS was set up as described in the Operation and Maintenance Manual (Bioprocess Control AB 2012). Figure 16 shows the AMPTS set-up.

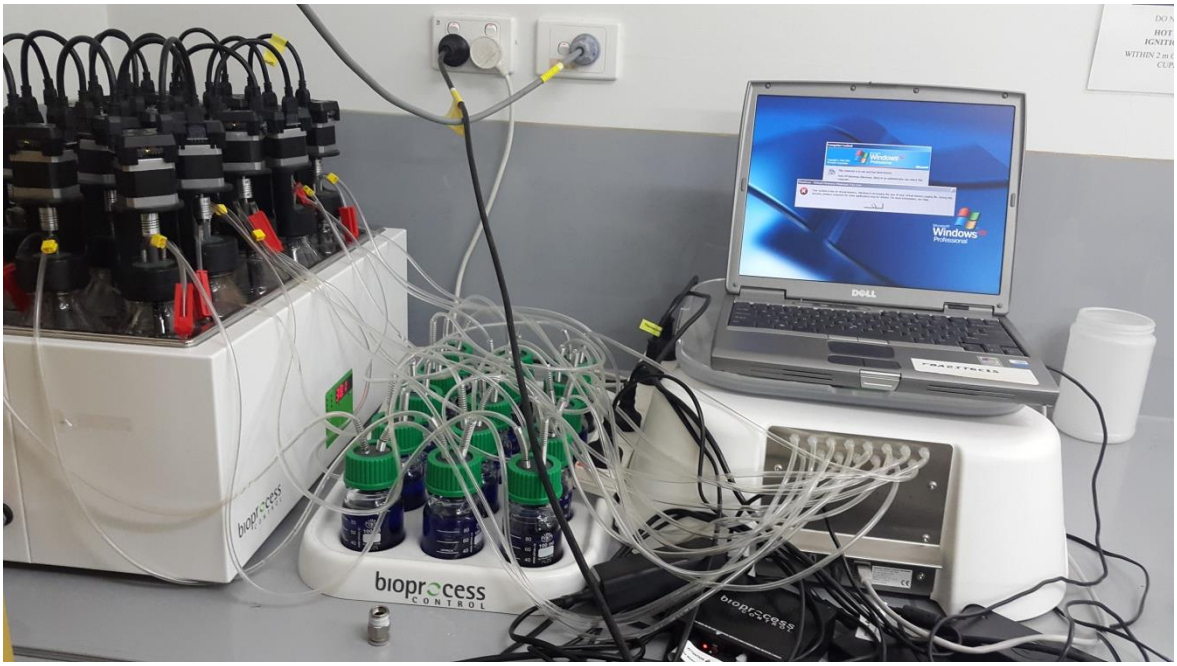


**Figure 16- AMPTS set-up**

Figure 17 shows an AMPTS bottle loaded with FOG. The bottle was tightly sealed to prevent any diffusion of gas.

**Figure 17- A loaded AMPTS bottle**

Prior to start of the experiment, 3M sodium hydroxide solution was prepared. 0.4% Thymolphthalein pH-indicator was prepared by adding 40 mg in 9ml ethanol 99.5%,

followed by addition of 1ml water. Sodium hydroxide (reagent grade 97%, Sigma–Aldrich) and 0.4% thymolphthalein pH indicator solution (dye content 95%, Sigma–Aldrich) are used to prepare 3M alkaline solution for the absorption of carbon dioxide (Bioprocess Control AB 2012). Anaerobic conditions during the preparation phase were generated by passing nitrogen gas through the system.

Two set of experiments were conducted for analyzing methane production at mesophilic temperature range (37ºC). In set 1, single substrate digestion experiments were performed. Inoculum obtained from the piggery farm and prepared bio-medium were added along with a substrate to each of the thirteen bottles of AMPTS set-up in set 1. Different inoculum to substrate ratios were used. Total volume in each of the bottles must not exceed 300ml (Bioprocess Control AB 2012). 100ml inoculum and 100ml bio-medium were added to each of the bottles. 50g of food waste was added to three bottles as triplicates, 50g FOG and 10g FOG were added to two bottles each and triplicates for 50g TWAS were used. S/I ratio for each of them is mentioned in the Table 5.

**Table 5- Set 1: To measure the methane production when individual substrate is added to the bottles for mesophilic temperature range**

| Bottle number | Substrate | Inoculum | Bio-medium | I/S ratio |
|---|---|---|---|---|
| 1 | 50g of FW | 100g of Inoculum | 100ml of bio-medium | 0.74 |
| 2 | 50g of FW | 100g of Inoculum | 100ml of bio-medium | 0.74 |
| 3 | 50g of FW | 100gof Inoculum | 100ml of bio-medium | 0.74 |
| | | | | |
| 4 | 50g of FOG | 100g of Inoculum | 100ml of bio-medium | 0.101 |
| 5 | 50g of FOG | 100g of Inoculum | 100ml of bio-medium | 0.101 |
| | | | | |
| 6 | 10g of FOG | 100g of Inoculum | 100ml of bio-medium | 0.51 |
| 7 | 10g of FOG | 100g of Inoculum | 100ml of bio-medium | 0.51 |
| | | | | |
| 8 | 50g of TWAS | 100g of inoculum | 100ml of bio-medium | 13.21 |
| 9 | 50g of TWAS | 100g of inoculum | 100ml of bio-medium | 13.21 |
| 10 | 50g of TWAS | 100g of inoculum | 100ml of bio-medium | 13.21 |
| | | | | |
| 11 | Control- 100g Inoculum + 100ml bio-medium | | | |
| 12 | Control- 100g Inoculum + 100ml bio-medium | | | |
| 13 | Control- 100g Inoculum + 100ml bio-medium | | | |

In set 2 of experiments, different combinations of substrates were co-digested in triplicates. All the substrates (in different proportions) were added to the bottles along with inoculum and bio-medium as shown in the Table 6.

**Table 6- Set 2: To measure the methane production and percentage of FOG which would be inhibitory for the combination of 3 substrates in a single bottle in mesophilic temperature range**

| Bottle number | Substrate | Inoculum | Bio-medium | I/S ratio |
|---|---|---|---|---|
| 1 | 50g FW + 25g FOG + 25g TWAS | 100g of inoculum | 100ml of Bio-medium | 0.16 |
| 2 | 50g FW + 25g FOG + 25g TWAS | 100g of inoculum | 100ml of Bio-medium | 0.16 |
| 3 | 50g FW + 25g FOG + 25g TWAS | 100g of inoculum | 100ml of Bio-medium | 0.16 |
| | | | | |
| 4 | 50g FW + 10g FOG + 25g TWAS | 100g of inoculum | 100ml of Bio-medium | 0.29 |
| 5 | 50g FW + 10g FOG + 25g TWAS | 100g of inoculum | 100ml of Bio-medium | 0.29 |
| 6 | 50g FW + 10g FOG + 25g TWAS | 100g of inoculum | 100ml of Bio-medium | 0.29 |
| | | | | |
| 7 | 25g FW + 10g FOG + 50g TWAS | 100g of inoculum | 100ml of Bio-medium | 0.37 |
| 8 | 25g FW + 10g FOG + 50g TWAS | 100g of inoculum | 100ml of Bio-medium | 0.37 |
| 9 | 25g FW + 10g FOG + 50g TWAS | 100g of inoculum | 100ml of Bio-medium | 0.37 |
| | | | | |
| 10 | 25g FW + 25g FOG + 50g TWAS | 100g of inoculum | 100ml of Bio-medium | 0.18 |
| 11 | 25g FW + 25g FOG + 50g TWAS | 100g of inoculum | 100ml of Bio-medium | 0.18 |
| 12 | 25g FW + 25g FOG + 50g TWAS | 100g of inoculum | 100ml of Bio-medium | 0.18 |
| | | | | |
| 13 | Control- 100g Inoculum + 100ml bio-medium | | | |
| 14 | Control- 100g Inoculum + 100ml bio-medium | | | |
| 15 | Control- 100g Inoculum + 100ml bio-medium | | | |

The second set of experiments helped in comparing:

- Methane produced in second set with its production when 50g of food waste was used in set 1
- Methane produced in second set with its production when 50g of food waste and 10g FOG were used in set 1
- Methane produced in second set with its production when 50g of TWAS and 10g FOG were used in set 1
- Methane produced in second set with its production when 50g of TWAS was used in set 1
- To investigate if combination involving 25g FOG produced more methane than 10g FOG

This set of experiment helped in determining if co-digestion with FOG produced more methane.

# CHAPTER 4: RESULTS

In this chapter, the characterisation analysis results and discussion, and AMPTS results for both the sets of experiments will be mentioned and discussed.

## 4.1 CHARACTERISATION ANALYSIS RESULTS

Table 7 shows the physical and chemical characterisation results obtained for inoculum, FW, FOG and TWAS.

**Table 7-Physical and chemical characterisation results**

| **Physical characterisation (Gravimetric analysis)** | | | | |
|---|---|---|---|---|
| Characteristic | Inoculum | FW | FOG | TWAS |
| Total solids (g/g substrate) | 0.11 ± 0.001 | 0.17 ± 0.004 | 0.96 ± 0.0004 | 0.009 |
| Volatile solids (g/g substrate) | 0.05 ± 0.002 | 0.14 ± 0.004 | 0.96 ± 0.0004 | 0.008 |
| Ash content (g/g substrate) | 0.05 | 0.03 | 0 | 0.0013 |
| Percentage of moisture | 89.4% | 83.1% | 3.8% | 99.1% |
| TVS/TS (%) | 47.6% | 80.23% | 100% | 85.7% |
| **Chemical characterisation** | | | | |
| Chemical oxygen demand (g/L) (1ml distilled water+1ml substrate) | 31.6 | 55.4 | NA | 4.36 |
| Chemical oxygen demand (g/L) (2ml of substrate) | NA | 48.68 | NA | NA |
| Chemical oxygen demand (g/L) (0.5ml of diluted substrate+1.5ml distilled water) | 27.15 | NA | NA | NA |
| Total organic carbon (Unfiltered and hydrolysed food waste) (g/L) | NA | 37.6 | NA | NA |
| Total organic carbon (Soluble substrate) (g/L) | NA | 14.23 | NA | 0.16 |
| Total nitrogen (Unfiltered and hydrolysed food waste) (g/L) | NA | 2.92 | NA | NA |
| Total nitrogen (Soluble) (g/L) | NA | 2.05 | NA | 0.06 |

Footnote: Total solids = mean ± standard deviation

Total volatile solids = mean ± standard deviation

NA: Not Applicable

## 4.2 CHARACTERISATION ANALYSIS DISCUSSION

### 4.2.1 Total solids and total volatile solids:

Total solids in the inoculum was 0.11g per gram inoculum. However, the volatile solids content in the inoculum was relatively lower than the volatile solids content published in other papers. Total volatile solids were 47.6% of the total solids. Li et al. (2011) conducted biogas production experiments using inoculum which had 70.9% volatile solids content in total solids. Wan et al. (2011) state the percentage of VS in the inoculum used. It was found that the percentage of VS/TS for inoculums used in their study was 69.5%. Luostarinen et al. (2009) mention that the inoculum used for anaerobic digestion experiments had 55% volatile solids.

A characterization study for the food waste obtained from a university cafeteria was conducted by Kwon et al. (2003). Their results show that VS/TS was 94% and moisture content was 80% and methane production was 440ml/g VS. Moisture content in the food waste used in the study conducted by Zhang et al. (2014) is 70-80%. Food waste used in the study conducted by Izumi et al. (2010) had VS/TS percentage as 94%. Kitchen waste used in the study by Li et al. (2013) had 86.3% of VS/TS and methane yield was 553 ml/g VS. The food waste used by Li et al. (2011) had 92.1% volatile solids in total solids. Methane production was 266ml/g VS in FW with S/I ratio of 0.52. Total volatile solids in the total solids in the food waste used in the present study were 80.23% of the TS.

Wang et al. (2013) conducted anaerobic digestion experiments using TWAS which had 78.9% VS/TS. Luostarinen et al. (2009) conducted anaerobic digestion study for sludge

which had percentage of VS/TS as 67%. TWAS used in the study by Wan et al. (2011) had 83.7% VS/TS. TWAS used in the present set of experiments has 85.7% VS/TS which was higher than the percentage stated in other two studies.

Percentage of VS/TS in FOG used by Wang et al (2013) was 99.9%. Li et al (2011) used FOG which also had a similar VS/TS percentage of 99.6%. Percentage of VS/TS in FOG used in the present set of experiments is 100%.

### 4.2.2 Chemical Oxygen Demand

Li et al. (2011) have mentioned the results for the anaerobic digestion study with total COD for the substrates used. The inoculum had COD value of 11.6g/L. COD for TWAS was 3.5g/L, for FW was 52.4g/L and for FOG, it was 375g/L. COD readings for the inoculum used in the current study was 31.6g/L, for FW was 55.4g/L, and for TWAS was 4.36g/L. The results were comparable.

### 4.2.3 Total Organic Carbon

Total organic carbon analysis was performed for hydrolyzed and unfiltered food waste as well as filtered food waste. It was found that TOC in total food waste (37599mg/L) was more than double the amount for soluble food waste (14225mg/L). Nitrogen content in unfiltered food waste was also very high in comparison to the soluble part of food waste.

### 4.2.4 C/N ratio

Zhang et al. (2014) mention that performance of AD is affected by C/N ratio as an optimum nutrient balance is essential for anaerobic bacteria and for maintaining a stable environment. Optimum amount of carbon is necessary for avoiding excessive ammonia inhibition. It is also stated that the optimum C/N ratio is generally considered to be within the range 20-30.

However, it was later found that the digestion process was effective at C/N ratios between 15 and 20. Also, optimum C/N ratio depends on the type of substrate and inoculum. C/N ratio for food waste used in the study by Li et al. (2013) was 20.3 and for the characterization study conducted by Zhang et al. (2006), C/N ratio was 14.8. C/N ratio in the food waste used in this investigation was 18.37. C/N ratio for TWAS used in the study conducted by Wan et al. (2011) was 6.7, which is higher than C/N ratio for the present study (2.8).

## 4.3 AMPTS RESULTS

### 4.3.1 Results for set 1 of experiments

Methane production from each of the substrates was calculated by taking the average of the results obtained from duplicate samples. Methane production plateaued after 63 days for FW, 15 days for 50g FOG, 37 days for 10g FOG, and 17 days for 50g TWAS. Table 8 shows the methane production results for set 1 experiment when single substrate was digested under anaerobic conditions. Table 9 shows the pH readings for the solutions in AMPTS bottles at the end of the first set of experiments.

**Table 8- Methane production from anaerobic digestion of individual substrate**

| Substrate | Methane production (Nml/ g VS of substrate) | Cumulative methane production (Nml) |
|---|---|---|
| Food waste (50g) | 673.7 ± 38.3 | 4634 ± 263 |
| Waste cooking oil (50g) | 4.2 ± 0.06 | 200 ± 3.2 |
| Waste cooking oil (10g) | 44.6 ± 2.6 | 429 ± 24.5 |
| Thickened waste activated sludge (50g) | 163.4 ± 50.5 | 63.7 ± 19.7 |

Footnote: Methane production = mean ± standard deviation

**Table 9- pH of substrates at the end of experiment 1**

| Substrate | pH (Average) |
|---|---|
| FW (50g) | 7.7 |
| FOG (50g) | 4.8 |
| FOG (10g) | 5.8 |
| TWAS (50g) | 7.6 |
| Control | 7.6 |

### 4.3.2 Discussion of results for set 1 of experiments

Methane yield from anaerobic digestion of food waste, FOG (Canola oil) and TWAS will be discussed in this section.

## ***Food waste as substrate***

Three duplicates were set up for methane production analysis from food waste. Mean cumulative methane production obtained was 673.7 ± 38.3 Nml/g VS of food waste after running the experiment for approximately 63 days. Figure 18 shows the cumulative methane yield (Nml/g VS) for the duplicates and their average.

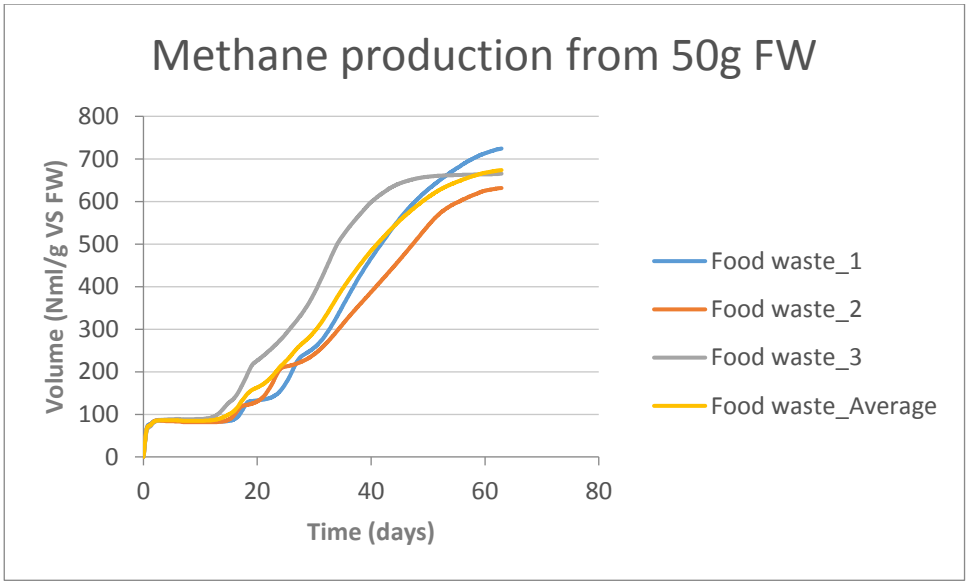


**Figure 18-Methane production (Nml/g of FW)**

Maximum amount of methane production was obtained in the first day (503.2mL/day and 73.1 mL/g VS/day). High production in the first few days is due to more balanced parameters-C/N ratio, I/S ratio and enzymes. However, after a day, there was a lag phase which continued for approximately thirteen days. After the lag phase, methane production was continuous. However, it started to plateau after 63 days. Methane production trend lines from the three duplicates were similar. The difference in the three duplicates was there because the food waste was not homogeneous and hence, it was difficult to obtain same duplicate and representative samples as explained by Cho et al. (1995). pH in the bottle was optimum (7.73).

Cho et al. (1995) also explain the reason for lag phase. Food waste is rich in high soluble organics, which rapidly get converted to volatile fatty acids at an early stage of digestion. This results in a drastic pH drop, which inhibits the initiation of methane fermentation with no sufficient buffering capacity. However, with time, the methane formers become acclimated by the buffer capacity compensation which occurs as a result of degradation of protein present in the food waste. The result of protein anaerobic degradation is ammonia

which buffers the volatile acids by maintaining elevated level of bicarbonate. Another reason for lag phase is lack of methanogens in the inoculum. The methanogens took around thirteen days to grow and acclimatize again. Moreover, food waste is not uniformly mixed in the AMPTS bottles. At some positions, more food waste can be accumulated in comparison to other positions in the bottles. This may lead to mobile methanogens reaching the accumulated food waste after some time, which can contribute to lag phase.

A recent study by Xie et al. (2017) report methane production of 652 ± 12 mL/g VS FW. I/S ratio used was 1.5. This result was comparable to the result obtained in the present study. However, I/S ratio used in the present study (0.74) was approximately half of the value used in this study (1.5). The lag phase reported was 0.46 ± 0.09 day whereas in the present study a longer lag phase of thirteen days was recorded. The ultimate methane yield reported was 2153 ± 39 ml whereas in the present study methane yield obtained was more than double, 4634 ± 263 ml. The experiment running time was just 14 days in comparison to 63 days in the present study.

Cho et al. (1995) conducted anaerobic digestion for Korean food wastes for investigating biochemical methane potential. Obtained methane yield was 472ml/g VS mixed food waste and the anaerobic biodegradability based on stoichiometric methane yield was 86%. Moisture content in the food waste was 74%. Percentage of VS/TS in food waste was 95%. C/N ratio was 16. The experiment duration was 33 days.

Another study was conducted by Lin et al. (2011) for anaerobic digestion of food waste from a Northern China city using a CSTR digester. Methane yield from food waste was found to be 560ml/g VS. VS/TS ratio was 0.93. C/N ratio in the food waste used was 17.2.

Zhang et al. (2006) conducted a study on food waste collected in the city of San Francisco, California. It was found that the methane yield was 435mL/g VS after 28 days of experiment. VS/TS ratio for the food waste was 0.85 and C/N ratio was 14.8. It was concluded that the food waste is desirable as a substrate for anaerobic digesters as it is highly biodegradable and has high methane yield potential.

Methane yield obtained in this study was more than the yield obtained in the other studies. The yield was high despite low VS/TS ratio in the food waste used in this study (80.23%) in comparison to 95% Cho et al. (1995), 92.5% Lin et al. (2011) and, 85.3% Zhang et al. (2006). Moreover, the percentage of VS/TS in the inoculum used in this study was 47.6%, which was low.

***FOG as substrate***

Long et al. (2012) report that FOG has a potential to produce substantial amount of methane due to high percentage of high strength organic content in it. Therefore, use of FOG in anaerobic digestion provides an economic incentive as it will lead to higher amounts of methane produced which in turn can be used to generate electricity. However, if FOG is used in high quantity, it will act as an inhibitory for methane production due to high lipid content. FOG causes inhibition for acetolastic and methanogenic bacteria. If used in full scale anaerobic digesters, FOG can cause substrate and product transport limitation, sludge floatation, digester foaming, blockages of pumps and pipes and gas collection and handling systems can be clogged. Kabouris et al. (2007) report that ultimate biodegradability of FOG is three times larger than WAS, and has a very high VS/ TS ratio (96.5% of VS in FOG in comparison to 65.7% in TWAS). Therefore, investigation of percentage of FOG which leads to a higher methane production and which leads to inhibition is a matter of interest.

Therefore, in this first set of experiments, different quantities of FOG were used-10g and 50g. It was found that 44.6 ± 2.6 ml methane/g VS FOG was produced when 10g FOG was used. 4.2 ± 0.06 ml methane/g VS FOG methane was produced when 50g of FOG was used. Methane production in case of 10g FOG stopped after 37.5 days whereas it stopped after 15 days when 50g FOG was used. It was found that pH level in 50g FOG bottles has dropped down to 4.83 whereas pH in case of 10g sample was 5.8.

High lipid content and drop in pH is the reason for brief period during which methane was produced and low yield of methane. Lay et al. (1999) stated that methane production from VFAs and lipids is optimal at a pH in the range 6.3 to 7.8. Long et al. (2012) explains that long chain fatty acids in FOG may have a detrimental effect on methanogenic bacteria when it is introduced at a high concentration. This explains very low methane production when 50g FOG was used whereas methane continued to be produced for a longer period when 10g FOG was used, as the concentration was relatively low. Bacteria get coated in a layer of LCFAs due to which cells access to the substrates is hindered and they lose their ability to produce methane. Therefore, LCFAs are toxic for bacteria. Hanaki et al. (1981) report that FOG may also retard the anaerobic digestion process. This damage to the cells is irreversible and the bacteria are not capable of building a tolerance to LCFA inhibition. However, Pereira et al. (2003) reported that even if LCFA can severely inhibit the methanogenic activity, anaerobic bacteria can still co-digest the adsorbed LCFA. Therefore, in the second set of experiments, FOG was used as a co-digestion substrate. Figures 19 and 20 show the methane production trends when 50g FOG and 10g FOG were used respectively. Two duplicates were used for both quantities of FOG.

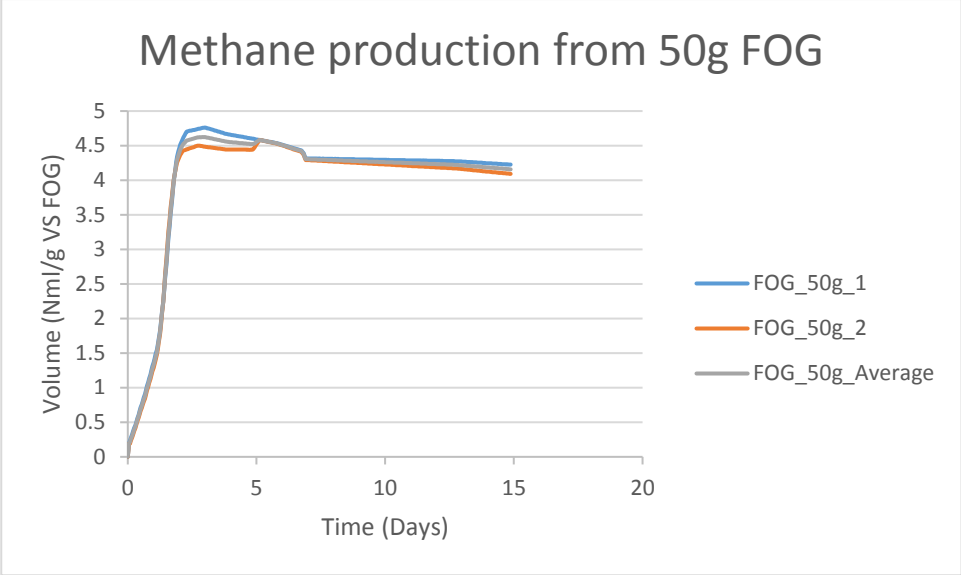


**Figure 19- Methane production (Nml/g of VS FOG (50g))**

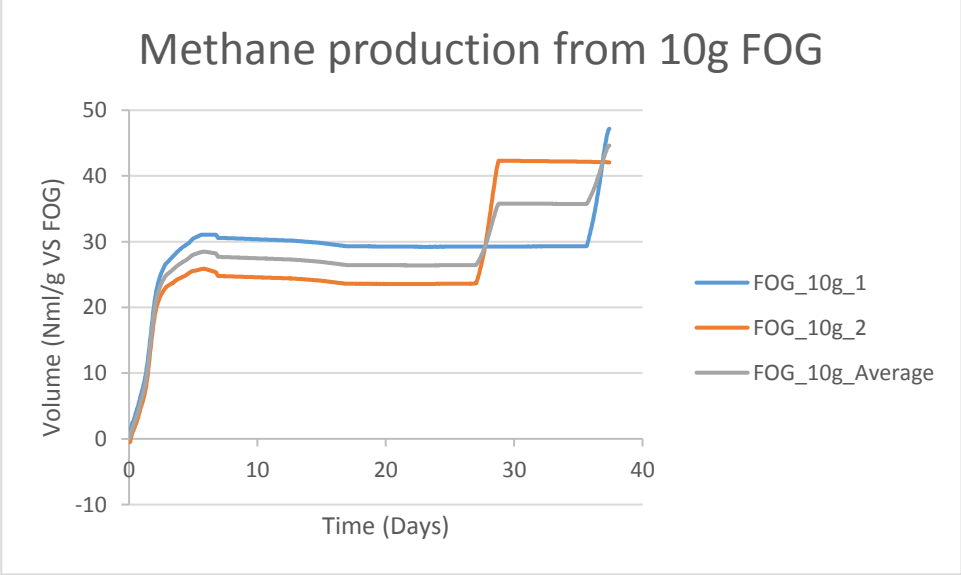


**Figure 20- Methane production (Nml/g of VS FOG (10g))**

Similar trends were obtained for methane production in case of duplicates. Some variations in methane production was because even if same amount of FOG was added to duplicates, the concentration of FOG in the bottles was observed not be uniform throughout as mixing

was not proper. This led to slightly different trends and amount of methane produced in duplicates. Also, more accumulation of LCFAs on bacteria in one of the bottles could have led to earlier end to methane production than observed in other bottle (duplicate) in case of 10g FOG. In case of 50g FOG, after 3 days, inoculum produced more methane than FOG, and that is why the graph (Figure 19) shows decreasing methane production.

Maximum methane production was observed on the second day of experiment in both the cases. 138.5mL/day and 12.9mL/g VS/day were recorded on the second day for 10g FOG and 147.7mL/day and 3.07mL/g VS/day were recorded on the second day for 50g FOG. High production in the first few days is due to more balanced parameters-C/N ratio, I/S ratio and enzymes. This balance may be disturbed with increasing time. There was a lag phase of approximately 30 days for FOG_10g_1 and 20 days for FOG_10g_2. Hanaki et al. (1981) report that LCFAs caused an increasing lag phase in methanogenic activity. This is one of the reasons of having a long lag phase in this study with FOG.

Other reason for low methane production in case of FOG was low I/S ratio (0.101) in case of 50g FOG and 0.5 in case of 10g FOG. FOG requires a high I/S ratio for digestion (Li et al. 2011). Therefore, more amount of inoculum was required. Also, it can be concluded from this study that more amount of bio-medium with FOG should have been used. Bio-medium contains ammonium carbonate which helps in maintaining the pH in the bottles and provides optimum and controlled conditions for methanogens. Low pH observed at the end of experiments proved that there was not sufficient bio-medium and hence, not optimum amount of ammonium carbonate in the bottles. However, difference of just one unit i.e. 4.8 in 50g FOG and 5.8 in 10g FOG shows that bio-medium has helped in maintaining pH to some extent. It can also be concluded that the bio-medium used by Owens et al. (1979) is not suitable for FOG when it is digested as a single substrate.

Li et al. (2011) conducted experiments with different I/S ratios for FOG. It was found that maximum methane production (383mL/g VS) took place when I/S ratio was 2. 63mL/g VS of methane was produced when I/S ratio was 1. However, no methane was produced when I/S ratio was 0.5 or 0.25. Wan et al. (2011) report that process stability could be negatively affected due to high concentration of FOG. It was also mentioned that FOG at a high concentration led to digestion failure due to acidification of digester. In the present study, 44.6 ± 2.6 mL/g VS of methane was produced when I/S ratio was 0.5 with 10g FOG. In contrast to the study conducted by Li et al. (2011) when no methane was produced with I/S ratio less than 0.5, 4.2 ± 0.06 mL/g VS methane was produced in the present study when I/S ratio was 0.101 with 50g FOG. This shows that FOG used in this study had a higher potential of generating methane than that used by Li et al. (2011).

From anaerobic digestion of FOG in the present study, it was found that FOG used in this study, which was Canola oil, is mainly non-biodegradable. This is evident from the low methane yield from 10g and 50g FOG. FOG was not toxic as the maximum methane production was observed in the first two days. However, due to its low bio-degradability, it is not suitable to be used as a substrate.

***TWAS as substrate***

Three duplicates were set up for methane production analysis from TWAS. Mean cumulative methane production obtained was 163.4 ± 50.5 Nml/g VS of TWAS after running the experiment for approximately 63 days. However, methane production from TWAS stopped after 17 days. Figure 21 shows the cumulative methane production for the duplicates and their average per gram VS.

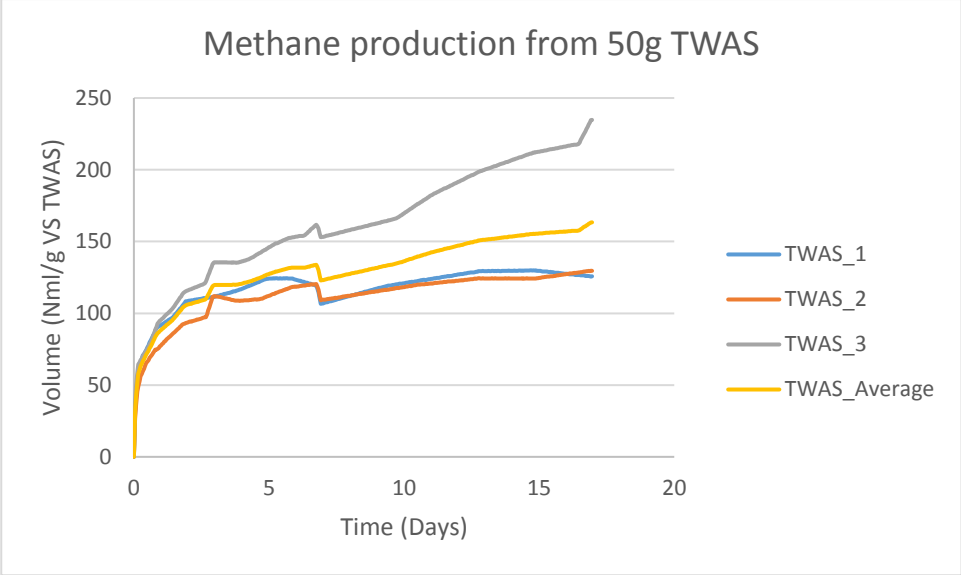


**Figure 21-Methane production (Nml/g of TWAS)**

Maximum amount of methane production was obtained in the first day (88.2 mL/g VS/day and 34.4 mL/day). However, it gradually slowed down over a week. The reason for maximum methane production in the first few days was more balanced C/N ratio, I/S ratio, enzymes, and other balanced parameters. These parameters may be disturbed with increasing time. There was no lag phase observed. TWAS provided adequate buffering, which prevented lag phase due to temporary acidification. It can be concluded that there was no toxic effect on methanogens in case of TWAS. Methane production trend lines from the three duplicates were similar. The difference in the three duplicates is there because the TWAS was not uniformly mixed in the AMPTS bottles and it was difficult to obtain same duplicate and representative samples as explained by Cho et al. (1995). It can be concluded that there was no toxic effect on methanogens in case of TWAS. pH in the bottle was optimum (7.67).

Davidson et al. (2008) state that typical methane potential of sludge is approximately 300-400 ml/g VS. Wan et al. (2011) also conducted a study on anaerobic digestion of TWAS. It was found that 252.4 ± 16.6 mL/gVS/day of methane was produced. I/S ratio used was 0.83. Another anaerobic digestion study on TWAS conducted by Chi et al. (2011) report methane yield of 230ml/g VS added.

Methane production from TWAS in the present study was less in comparison to that obtained in the other studies. Zhang et al. (2014) mention that performance of anaerobic digestion is affected by C/N ratio, as an optimum nutrient balance is essential for anaerobic bacteria and for maintaining a stable environment. C/N ratio for TWAS used in the present study was 2.88. C/N ratio for TWAS used in the study conducted by Wan et al. (2011) was 6.7, which is higher than C/N ratio for the present study. This was a reason for low methane production from TWAS.

## ***Conclusion***

It was observed that maximum methane production was obtained when food waste was digested, followed by TWAS, FOG_10g and finally FOG_50g. Maximum lag phase was when FOG was digested, followed by FW. No lag phase was observed in TWAS digestion. It was found that higher the content of FOG (Canola oil), lower is the methane yield. It can be concluded that FOG is not a suitable substrate due to its low biodegradability which leads to low methane yield. It was also found that FOG was not inhibitory. Table 10 summarises the results obtained from this study and compares them with the results obtained for other studies in which FW, FOG and TWAS were used as single substrates.

**Table 10- Comparison of methane yield**

| Substrate | I/S | Methane yield (ml/g VS substrate) | Ultimate methane yield (ml) | Lag phase (Days) | Methane yield (ml/g VS substrate/day) ($R_m$) | Maximum Methane yield (ml/day) | Reference |
|---|---|---|---|---|---|---|---|
| 50g FW | 0.74 | 673.7±38.3 | 4634±263 | 13 | 73.2 | 503.2 | Present study |
| | 1.92 | 266 | | | | | Li et al. (2011) |
| | 0.97 | 308 | | | | | Li et al. (2011) |
| | 0.49 | 33 | | | | | Li et al. (2011) |
| | 1.5 | 652±12 | 2153±39 | 0.46±0.09 | | 807±66 | Xie et al. (2017) |
| 50g FOG | 0.101 | 4.2±0.06 | 200±3.3 | | 3.07 | 147.7 | Present study |
| 10g FOG | 0.51 | 44.6±2.6 | 429±24.5 | 25 | 13.6 | 131 | Present study |
| | 2 | 383 | | | | | Li et al. (2011) |
| | 1 | 63 | | | | | Li et al. (2011) |
| | 0.5 | 0 | | | | | Li et al. (2011) |
| 50g TWAS | 13.21 | 163.4±50.5 | 63.7±19.7 | | 88.2 | 34.4 | Present study |
| | 0.83 | | | | 252.4±16.6 | | Wan et al. (2011) |
| | | 180 | | | | | Wang et al. (2013) |
| | | 300-400 | | | | | Davidson et al. (2008) |
| | | 230 | | | | | Chi et al. (2011) |
| | | 217 | | | 180±0.01 | | Jang et al. (2013) |

Footnote: Methane yield = mean ± standard deviation

### 4.3.3 Results for set 2 of experiments

Second set of experiments involved methane production from different combinations of substrates. Duration of the experiment was 48 days. Table 11 shows the methane production results for set 2 experiments.

**Table 11- Methane production from anaerobic co-digestion**

| | Substrate | Methane production (Nml/ g VS of substrate) | Cumulative methane production (Nml) |
|---|---|---|---|
| Case 1 | 50g FW_25ml FOG_25g TWAS | 66.2 | 468.2 |
| Case 2 | 50g FW_10ml FOG_25g TWAS | 311.2 | 2201.4 |
| Case 3 | 25g FW_10ml FOG_50g TWAS | 669.7 | 2564.7 |
| Case 4 | 25g FW_25ml FOG_50g TWAS | 219.6 | 841.1 |

Methane production plateaued after 16 days for case 1 (50g FW_25g FOG_25g TWAS) whereas it continued to be produced in other cases. Figure 22 shows the methane production from different combinations of substrates.

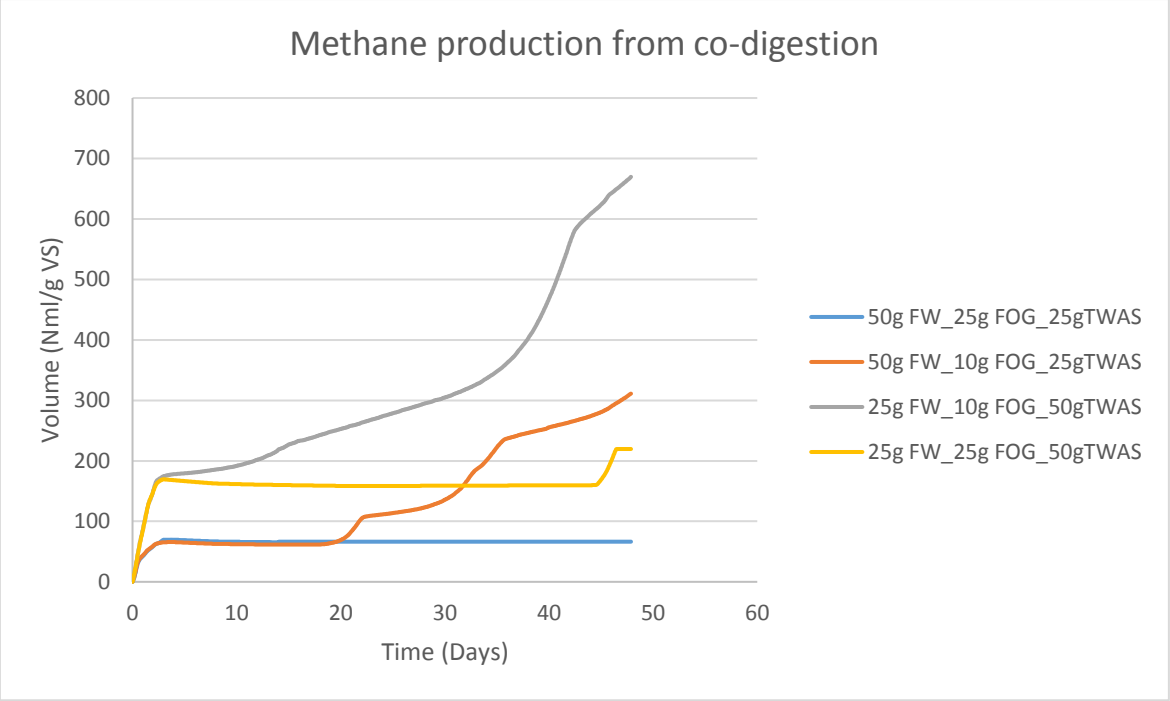


**Figure 22- Methane production from co-digestion**

### 4.3.3 Discussion of results for set 2 of experiments

In the following section, results for methane production from co-digestion will be discussed.

***50g FW_25g FOG_25g TWAS***

Methane production obtained from this case was 66.2 Nml/g VS after running the experiment for approximately 48 days. However, methane production stopped after 16 days in this case. Also, methane yield in this case was the lowest. From set 1 of experiments, it was established that FOG is mainly non-biodegradable as it produced very low yield of methane. This case re-affirmed that due to high proportion of FOG, methane yield was negatively affected. Percentage of FOG in this case was 77.3% of the total VS load.

Methane production from FW in first set of experiments was 673.7 ± 38.3 Nml/g VS FW whereas with 50g present in this case, methane produced was drastically reduced due to co-digestion with FOG. It was observed that FOG had accumulated at the top of the solution in the AMPTS bottles. Methane produced in the process could not escape properly in the gas phase due to this scum layer formation. Also, Long et al. (2012) explain that bacteria get coated in a layer of LCFAs due to which cells access to the substrates is hindered and they lose their ability to produce methane. This is evident by low methane yield obtained in this case.

***50g FW_10g FOG_25g TWAS***

Methane production from this case was 311.2 Nml/g VS. As shown in the figure 22, lag phase observed in this case continued from 4 days to 20 days. Production increased for the next couple of days. This was followed by a period of five days when rate of methane production was low. The rate increased again in the following days. The lag phase or low rate of methane production can be explained from the toxic effect of FW and FOG. Toxic effect could have been because of temporary acidification due to FW as explained by Cho et al (1995). pH drops due to lack of sufficient buffering capacity. However, with time, the methane formers become acclimated by the buffer capacity compensation due to degradation of protein present in the food waste. Lag phase was also observed due to the same reason when 50g FW was digested.

Due to lower content of FOG in this case, nearly 4.5 times more methane yield was observed than that in case 1. However, methane production was still less than that obtained when 50g FW was digested individually, which could be explained by presence of FOG. Percentage of FOG in this case was 57.6% of the total VS load. FOG thickened the solution due to

which the mixing in the AMPTS bottles was improper. Substrates and microbes got coated with a layer of oil which led to mass transfer problems.

***25g FW_10g FOG_50g TWAS***

Methane yield from this proportion of substrates was the highest (669.7 Nml/g VS). There was no lag phase observed which is consistent with the result obtained from anaerobic digestion of 50g TWAS in first set of experiments. This indicated that TWAS has no toxic effect on the digestion process. Benefit of co-digestion was slightly observed in this case. More methane yield was observed than that obtained when 50g TWAS was digested in set 1. The methane yield is close to the yield obtained when 50g FW was digested individually (which was the highest in set 1 experiment).

More methane was produced in this case in comparison to case 2, even with a higher percentage of FOG in the VS load (71.6%), as the solution in the AMPTS was not as thick as it was in case 2. TWAS was in the liquid form whereas FW was slurry. Due to dilution, better mixing was observed in this case, which led to more efficient transfer of substrates to the microbes and as a result, more methane yield was observed. Higher methane yield was obtained from this case than from case 4 which re-affirms that high proportion of FOG lowers the methane yield. (10g FOG in this case versus 25g FOG in case 4).

***25g FW_25g FOG_50g TWAS***

219.6 Nml/g VS methane was produced in this case, which is the second lowest methane yield in set 2. Low yield could be explained by high proportion of FOG (Long et al. 2012). FOG was accumulated at the top of the solution, as was observed in case 1. There was a long lag phase of 42 days. The lag phase could be due to high content of FOG (86.3% of the total

VS load). Hanaki et al. (1981) report that LCFAs caused an increasing lag phase in methanogenic activity. The lag phase could also be explained by lack of mixing in the AMPTS bottles and formation of scum layer of FOG at the top of the solution. Accumulated methane in the bottles could not escape in the gaseous form due to the scum layer. Co-digestion did not increase methane yield even in this case.

It was determined that FOG did not cause inhibition even when it constituted 86.3% of the total VS load. More methane yield was observed than in case 1 as the solution in the AMPTS bottles was not as thick as it was in case 1. As a result, mixing in the bottles in this case was better. Methane produced could escape the bottle more easily than in case 1.

## ***Conclusion***

25g Food Waste_10ml FOG_50g TWAS (Case 3) came out as the best proportion for maximum methane yield. However, as proposed, in previous studies, by Grosser et al. (2017), Li et al. (2011), Alqaralleh et al. (2016), Xie et al. (2017) that co-digestion enhances the methane production, increased methane yield was not observed with co-digestion in this study, except slight enhancement in case 3 with co-digestion.

It was established by set 1 experiments that FOG gives a low methane yield. Low yield in set 2 experiments due to presence of FOG re-affirmed that it is not a suitable substrate for methane production. This is contradiction with the results obtained by Long et al. (2012), who report that FOG has a potential to produce substantial amount of methane due to high percentage of high strength organic content in it. This is also in contradiction with the results obtained from the study conducted by Kabouris et al. (2009), who mention that when a high quantity of FOG (48% of total VS load) was co-digested with municipal sludge, it led to 2.95 times larger methane yield than that obtained by anaerobic digestion of sludge. Another

study conducted by Wang et al. (2013) showed that co-digestion of grease inceptor waste (which contained FOG) with municipal sludge increased methane yield by 4.2 times than obtained by digestion of sludge, and corresponding percentage of FOG which led to highest methane yield was 65.5% (w/w). In contrast, FOG lowered methane yield even when present at 57.6% in the co-digestion experiments in the present study.

In the present study, FOG did not inhibit the methane process. This is evident from instant methane production in set 1 as well as in all four cases of co-digestion (set 2), and even 50g of FOG in set 1 produced methane. It was not inhibitory even when it constituted 86.3% of the total VS load (case 4). However, due to low biodegradability of FOG, low yield of methane was obtained in set 1 experiment. In co-digestion experiment (set 2), along with low bio-degradability, other problems arose due to FOG:

- Lack of proper mixing in the AMPTS bottles. The substrates were not uniformly mixed which led to low methane yield. Access of substrates to microbes was difficult.
- FOG was accumulated at the top of the surface of the solution in the AMPTS bottles as shown in Figure 23. This led to formation of a scum layer. It was difficult for methane produced and accumulated in the AMPTS bottles to escape in the gaseous form due to this layer.
- Combination of all the substrates and inoculum led to formation of a thick solution. Due to high thickness, mass transfer of substrates to the microbes was improper.
- FOG coated the bodies of microbes as well substrates.

**Figure 23- FOG accumulation on the top surface of solution in AMPTS bottle**

These reasons explain the maximum methane yield from 25g FW_10ml FOG_50g TWAS (Case 3), followed by 50g FW_10ml FOG_25g TWAS (Case 2) , 25g FW_25ml FOG_50g TWAS (Case 4), and the least methane yield from 50g FW_25ml FOG_25g TWAS (Case 1). Also, above problems did not allow enhancement of methane production in co-digestion experiment than that obtained in anaerobic digestion of single substrates. Therefore, to obtain high methane yield, anaerobic digestion of FOG (Canola oil), which is mainly non-biodegradable, must be avoided.

# CHAPTER 5: CONCLUSIONS

## 5.1 CONCLUSIONS

The project investigated methane production by anaerobic co-digestion of food waste, fats, oil and grease and thickened waste activated sludge using Automatic Methane Potential Test System. Two sets of experiments were conducted. In the first set, the substrates FW, FOG and TWAS, were anaerobically digested individually. In the second set, they were combined in different proportions and were digested to investigate the most optimum combination and if co-digestion increases the methane production. Special emphasis was given to the percentage of FOG which could be inhibitory. All the experiments were carried out at mesophilic temperature range (37$^{o}$C).

Food waste and waste cooking oil (FOG) used in the study were obtained from the University of Southern Queensland Refectory, Toowoomba, Australia. The food waste comprised of a mixture of chips, bacon, fruits and their peals, and bread. It was grinded to form a slurry. FOG used in this study was mainly Canola oil. Thickened waste activated sludge was obtained from the Wetalla Wastewater Treatment plant in Toowoomba. The inoculum was obtained from the pond at a piggery farm located in Lockyer Valley in Queensland, Australia. A bio-medium, which provides essential micro-nutrients and macro-nutrients to microbes, was prepared and added to each of the AMPTS bottles at the start of experiments along with substrates and inoculum.

In the first set of experiments, maximum methane yield was obtained from 50g FW (673.7 ± 38.3 Nml/g VS FW), followed by 50g TWAS (163.36 ± 50.49. Nml/g VS TWAS). 10g FOG produced 44.63 ± 2.55 Nml/g VS FOG whereas 50g FOG generated just 4.16 ± 0.06 Nml/g VS FOG. Methane production plateaued after 63 days for food waste, 15 days for

50ml waste cooking oil, 37 days for 10ml waste cooking oil, and 17 days for TWAS. Maximum methane production was observed in the first day for FW and TWAS and second day with FOG. This was because of more balanced C/N ratio, I/S ratio and enzymes in the beginning.

Lag phase of 13 days was observed in case of FW, which occurred due to temporary acidification due to formation of volatile fatty acids. There was a lag phase of approximately 25 days (average) for both the duplicates in case of 10g, which occurred as LCFAs caused an increasing lag phase in methanogenic activity (Hanaki et al. 1981). There was no lag phase observed with TWAS. C/N ratio was optimum in case of FW (18.37). However, it was low in TWAS which led to lower methane yield (2.88).

From the first set of experiments, it was established that FOG (Canola oil) is not a suitable substrate. It is highly non-biodegradable, which is evident from low yield of methane. It was found that higher the content of FOG, lower is the methane yield. Low I/S ratio- 0.101 in case of 50g FOG and 0.5 in case of 10g FOG was another reason for low methane production in case of FOG. Therefore, more amount of inoculum was required. Also, it can be concluded from this study that more amount of bio-medium with FOG should have been used. Low pH (4.8 in case of 50g FOG and 5.8 for 10g FOG) observed at the end of experiments proved that there was not sufficient bio-medium.

From the second set of experiments it was found that maximum methane yield was obtained from Case 3- 25g FW_10g FOG_50g TWAS (669.7 Nml/g VS), followed by Case 2- 50g FW_10g FOG_25g TWAS (311.2 Nml/g VS), and Case 4- 25g FW_25g FOG_50g TWAS (219.6 Nml/g VS). Least amount of methane was generated from Case 1- 50g FW_25g

FOG_25g TWAS (66.2 Nml/g VS). It was found that co-digestion did not increase methane yield in comparison to individual substrate digestion, except slight enhancement in Case 3.

It was re-affirmed in the second set of experiments that FOG (Canola oil) is not a suitable substrate for methane production by low yield of methane. Low methane yield was observed even when FOG was present at 57.6% of the total VS load (case 2). FOG did not cause inhibition of anaerobic digestion process even when 86.3% of the total VS load was from FOG (case 4). However, it led to some problems which led to reduction in methane yield. These problems included accumulation of FOG at the top surface of the solution in AMPTS bottles due to which the methane produced could not escape in the gaseous form, and coating of oil on microbes' bodies and substrates. Other problems included lack of proper mixing in the bottles, and formation of a thick solution which included all the substrates, inoculum, and bio-medium. This led to improper mass transfer of substrates to microbes and microbes could not acquire the nutrients and sufficient food. As a result, co-digestion did not provide better methane yield than single substrate anaerobic digestion.

## 5.2 RECOMMENDATIONS

From both set of experiments, it was established that FOG (Canola oil) is not a suitable substrate for anaerobic co-digestion due to its low biodegradability. However, it can be further investigated if the yield can be improved if a higher I/S ratio and more bio-medium is used with FOG. Better mixing in AMPTS bottles could also lead to a higher methane yield. Thick substrate and inoculum solution prevents proper mass transfer from substrates to microbes, hence, thick solutions must be avoided if the experiment is carried out using AMPTS. Canola oil used in this study did not inhibit the digestion process but reduced the methane yield. However, use of other type of waste cooking oil may give different results. Therefore, investigation with other type of oil may be useful.

There was no lag phase observed in anaerobic digestion of TWAS and hence, it is a useful substrate. Food waste has a high potential of generating methane. Therefore, anaerobic digestion plants may digest food waste and sludge for generating methane. It will be an efficient source of renewable energy generation and utilization of excess amount of waste produced in the world.

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# APPENDIX A – PROJECT SPECIFICATION

| | |
|---|---|
| FOR: | Prof. Karu Karunasena |
| TOPIC: | Investigation of methane production by anaerobic co-digestion of food waste, fats, oil & grease, and thickened waste activated sludge using Automatic Methane Potential Test System |
| SUPERVISORS: | Dr. Antoine Trzcinski |
| ENROLMENT: | Master of Engineering Science (Environmental) (On-campus) |
| PROJECT AIM: | The project aims to investigate methane production by anaerobic co-digestion of food waste, thickened waste activated sludge, and oil & grease using Automatic Methane Potential Test System. The project also aims to investigate the stability of the digestion process at laboratory scale if high proportion of oil and grease is present in the feedstock. |
| SPONSORSHIP: | ……. |
| **PROGRAMME:** | Master of Engineering Science (Environmental) |

1. Research the background literature on anaerobic digestion of food waste, thickened waste activated sludge, and oil & grease.
2. Characterisation of food waste, thickened waste activated sludge, and oil & grease: Measurement of Total solids, Volatile solids, Total organic carbon, Chemical Oxygen Demand, Nitrogen, and ash content
3. Conduct co-digestion experiments using Automatic Methane Potential Test System to investigate methane production. Food waste, waste cooking oil, and thickened waste activated sludge will be anaerobically digested in two sets of experiments. In the first set, all three substrates will be digested individually, whereas in the second set, these substrates will be combined in different proportions and co-digested to find out the most optimum combination. Special emphasis will be given on the proportion of fats, oil and grease.
4. Analyse the results obtained at the end of the experiments.
5. Submit an academic research dissertation.

AGREED:

Nikita Bahri (Student) _____ (Supervisor)

Date: 20/03/2017 Date: 20/03/2017

# APPENDIX B – RISK ASSESSMENT

Risks are involved while conducting engineering experiments/work. Therefore, control strategies were identified and applied while conducting experiments.

In this project, work with chemicals was involved and risks involved for every chemical was different. Use of chemicals with attention and precautions was essential. All the risks associated with the project work, hazards, their likelihood as well as the control strategies are listed in the Table 11.

**Table 11- Risk assessment chart**

| | Risk | Hazard | Likelihood | Control |
|---|---|---|---|---|
| 1 | Handling Sodium hydroxide (NaOH) | Corrosive if inhaled; Can cause burning sensation, pain, redness if comes in contact | Unlikely | Handle with care; Use of safety goggles, lab coat, gloves; good ventilation |
| 2 | Carrying Lithium battery | Combustible gas release, corrosive electrolyte release, fire | Rare | Managing fire and fumes emissions; neutralizing electrolyte spillage |
| 3 | Handling electrical devices close to water | Electric chock | Unlikely | Handling the equipment with precautions, not using wet hands |
| 4 | Handling sodium sulphide | Formation of hydrogen sulphide | Unlikely | Carrying out the experiment in fume hood, Wearing safety goggles |
| 5 | Handling nitrogen gas | Nitrogen can act as asphyxiate; Nitrogen cylinder may fall | Rare | Carrying out the experiment in well ventilated area; Nitrogen cylinder must be attached to the bench or trolley so that it does not fall off |

| | | | | |
|---|---|---|---|---|
| 6 | $K_2HPO_4$ | Can cause skin irritation | Unlikely | Use of gloves, lab coat, safety goggles |
| 7 | $NaH_2PO_4 \cdot 2H2O$ | Can cause skin irritation | Possible | Use of gloves, lab coat, safety goggles |
| 8 | Resazurin | Can cause skin irritation | Possible | Use of gloves, lab coat, safety goggles |
| 9 | $FeCl_2 \cdot 4H2O$ | Can cause skin irritation, damage to eyes | Unlikely | Use of gloves, lab coat, safety goggles |
| 10 | $MnCl_2 \cdot 4H2O$ | Can cause skin irritation, damage to eyes | Unlikely | Use of gloves, lab coat, safety goggles |
| 11 | $CoCl_2 \cdot 6H2O$ | In case of skin contact (irritant), of eye contact (irritant), of ingestion, of inhalation<br><br>(lung irritant) | Unlikely | Use of gloves, lab coat, safety goggles |
| 12 | $ZnCl_2$ | In case of skin contact (corrosive, permeator), of eye contact (corrosive) | Unlikely | Use of gloves, lab coat, safety goggles |
| 13 | $CuCl_2 \cdot 2H_2O$ | In case of skin contact (permeator). Corrosive to eyes and skin. | Unlikely | Use of gloves, lab coat, safety goggles |
| 14 | $AlCl_3 \cdot 6H_2O$ | In case of skin contact (corrosive, permeator), of eye contact (corrosive) | Unlikely | Use of gloves, lab coat, safety goggles |
| 15 | $Na_2MoO_4$ | Redness on skin, redness in eyes, cough if inhaled | Unlikely | Use of gloves, lab coat, safety goggles |
| 16 | $NiCl_2 \cdot 6H_2O$ + 1 ml HCl | In case of skin contact (corrosive, permeator), of eye contact (corrosive) | Unlikely | Use of gloves, lab coat, safety goggles |
| 17 | $NH_4HCO_3$ | In case of skin contact (corrosive, permeator), | Unlikely | Use of gloves, lab coat, safety goggles |

| | | | | |
|---|---|---|---|---|
| | | of eye contact (corrosive | | |
| 18 | $NaHCO_3$ | Can cause mild eye irritation. May cause respiratory tract irritation, if inhaled | Possible | Use of gloves, lab coat, safety goggles |
| 19 | $Na_2S \cdot 9H_2O$ | In case of skin contact (corrosive, permeator), of eye contact (corrosive) | Unlikely | Use of gloves, lab coat, safety goggles |
| 20 | $CaCl_2 \cdot 2H_2O$ | Can cause serious eye irritation | Unlikely | Use of gloves, lab coat, safety goggles |
| 21 | $MgCl_2 \cdot 4H_2O$ | Can cause mild eye irritation. May cause vomiting, nausea if inhaled | Possible | Use of gloves, lab coat, safety goggles |
| 22 | Sulphuric acid | Very hazardous in case of skin contact (corrosive, irritant, permeator), of eye contact (irritant, corrosive), of ingestion, and of inhalation | Unlikely | Use of face mask, gloves, lab coat, safety goggles. Using it in the fume hood. |
| 23 | Handling sludge | Contract a disease | Rare | Use of gloves, lab coat, N95 mask and safety goggles. Get vaccination for Typhoid, Hepatitis A and B |

# APPENDIX C – PROJECT RESOURCE REQUIREMENT

The project resource request was for the necessary vaccines required for handling TWAS and inoculum. The details are mentioned below.

*Faculty of Health, Engineering and Sciences*

**MASTERS PROJECT RESOURCE REQUEST**

**For ENG8411/8412/8414/8002 Courses**

This form must be typed and completed in full.

| | |
|---|---|
| Student Name: | **Nikita Bahri** |
| Student Number: | **0061082104** |
| Program/Major/Course:<br>(eg. MENS/Civil/ENG8411) | **MENS/ Environmental/ENG8411** |
| Project Title:<br>(Full title needed) | **Investigation of methane production by anaerobic co-digestion of food waste, thickened waste activated sludge & FOG using Automatic Methane Potential Test System** |
| Principal Supervisor: | **Dr. Antoine Trzcinski** |
| Funds requested:<br>(Max $200/per project ) | **$265** |

**Please provide a brief description of what is to be purchased (attach a page if space is not sufficient) and an itemised price list (if you want to purchase more than one item). The total cost should equal the amount requested.**

**Vaccinations required – from USQ Student Services**

**3 doses of Hepatitis A & Hepatitis B vaccine - $210 ($70 each)**

**Typhoid Vaccine - $55**

I confirm that these funds are essential to successfully complete this project.

**Principal Supervisor:** _____ **Date:** 22/06/2017

Signature

**Approved By : Dr. Andreas Nataatmadja:** _____ **Date:** 22/06/2017

(Examiner – ENG8412, Assistant Examiner – ENG8411/8414/8002)

| | |
|---|---|
| **Project Resource Number** | **204010-00-1002457** |

**Approved by HES – Finance:** _____ **Date:** _____

The Project Resource Number is allocated and budget total confirmed as above. The student must quote this number on every request for project parts and on every workshop job form. The Examiner/Assistant Examiner will then endorse these forms and pass on to the supervisor for further action. Supervisors, please arrange purchase through HES – Finance. Note that there is no mechanism for students to initiate purchases in advance and then seek reimbursement.

*USQ collects personal information to assist the University in providing tertiary education and related ancillary services and to be able to contact you regarding enrolment, assessment and associated USQ services. Personal information will not be disclosed to third parties without your consent unless required by law.*

ENG8411 & ENG8412 RESOURCE REQUEST FORM VALID AT: 22 JUNE 2017 ISSUED 28/2/2014